Metabolic rates and feeding behaviour of sprat, *Sprattus sprattus* L.

Dissertation

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Summary

European sprat (*Sprattus sprattus* L.) is a small pelagic clupeid species with a wide distribution in shelf areas of the Northeast Atlantic. Especially in the Baltic Sea sprat is an ecological key species serving on the one hand as main prey for Baltic cod and on the other hand being the most abundant planktivorous fish species. Previous quantifications of the top down controls of the large sprat stocks, however, have to be regarded as preliminary due to the limited and disputed data on sprat daily rations. The general aim of the present thesis was to investigate physiological and behavioural attributes relevant for the ongoing development of bioenergetics growth and population models for Baltic Sea sprat.

As a central component of a bioenergetics model for sprat, the effects of temperature and body mass on metabolic rates were investigated (**Manuscript 1**) by measuring oxygen consumption rates (M_{O2}; $mgO_2\ fish^{-1}h^{-1}$) of sprat (3.1-9.7 g wet weight) in an intermittent-flow respirometer over nine different temperatures between 9 and 21°C. Standard metabolic rates (R_S) as calculated from the 10% percentiles of repeated measurements were on average 12% lower than routine metabolic rates (R_R) and still influenced by continuous swimming activity. Metabolic rates increased exponentially with temperature (Q_{10} = 2.2), similarly as reported for other clupeoids (Q_{10} = 1.8-2.2), but the metabolic scaling exponent was higher (b = 1.073) than typically found exponents of b ~ 0.8. This is most likely a consequence of the permanently elevated activity level in sprat. The high permanent swimming activities and the little difference between R_S and R_R indicated that the concept of standard metabolism may not be meaningful in schooling planktivorous fish. Since swimming has in general a great impact on fish metabolism, the costs for spontaneous swimming patterns in sprat were determined from respirometry experiments with simultaneous image recordings of fish (**Manuscript 2**). Therefore only measurements obtained in the acclimation periods (shortly after transfer into the respirometer) were used, because here swimming activities and metabolic rates were more variable than during later routine phases. An automated fish-tracking program was applied in order to detect different movement patterns like mean swimming speeds and turning rates from simultaneous image recordings. Spontaneous swimming was dominated by low swimming speeds (~ 0.57 body length s^{-1}) and frequent sharp turns (>90°), which could be related to mean oxygen consumption rates. These swimming patterns were related to mean oxygen consumption rates of the fish groups by a nonlinear model. Temperature (10-19°C) had no direct effect on the performed swimming speeds or turns>90°, but M_{O2} showed a greater increase with swimming speed at higher temperatures. The final model for the energy costs of spontaneous activity includes mean horizontal swimming speeds and turns>90° and can be used to approximate for swimming costs when the frequency of such swimming patterns is known.

So far no information information on feeding rates in relation to prey densities is available for sprat, although this is essential for the evaluation of maximum consumption rates. Sprat and herring (*Clupea harengus* L.) are directly competing for the same food

resources, especially during the juvenile phase when both clupeids school together in shallow coastal regions. However, so far no comparative studies were undertaken to investigate particulate feeding rates of these two species. Thus, laboratory feeding experiments were conducted in order to investigate the relationship between particulate-feeding rates and prey concentrations (1-160 l^{-1}) of juvenile sprat and herring (**Manuscript 3**). Thereby two different single prey types were used, which differed in their escape response to predator presence. It was assumed that feeding on non-evasive *Artemia* nauplii reflect maximum possible feeding rates comparable to feeding on cladocerans in the natural diet, whereas experiments with *Acartia tonsa* (copepoda: calanaoida) allowed determining the feeding rate at a natural prey organism with high escape abilities. While herring were occasionally filter-feeding at prey concentrations >50 l^{-1}, were sprat strictly sticking to particulate feeding mode. It was for the first time systematically evaluated that sprat is an obligate particulate feeder and in contrast to herring not able to switch to filter-feeding. Both fishes showed a type-II functional response (asymptotically decelerating intake rates with prey densities) with lower feeding rates when fish preyed on *A. tonsa* compared to experiments with *Artemia*. During feeding on copepods, both fishes showed an S-shaped deformation of their body before the biting attack, which is related to the escape response of *A. tonsa*. Functional response parameters were not significantly different between sprat and herring, despite at copepods concentrations below ~40 l^{-1}, where herring could achieve higher biting rates than sprat. These results enhance the understanding of the interaction of plankton populations and competing planktivorous fish species. The parameters of the functional response curves can improve fish bioenergetics models implemented into end-to-end ecosystem models. However, since a realistic model framework must include estimates of swimming patterns in relation to prey densities, the above mentioned feeding experiments were also used to investigate foraging related swimming patterns in sprat and herring (**Manuscript 4**). Therefore, swimming patterns and behavioural components were analysed from digital images of a top camera (for horizontal components) as well as from underwater images (for vertical components). Both fish species increased their horizontal swimming speeds (cm s^{-1}; body length s^{-1}), turning rates (°s^{-1}), numbers of sharp turns (>90°) and accelerations with prey concentrations. For these swimming patterns nonlinear (Gompertz-function) mixed-effects models were applied, which considered differences between fish species and prey types and could take into account that repeated measurements with the same fish group were conducted. Overall were the maximum values of all horizontal swimming patterns significantly lower when fish were feeding on copepods instead of *Artemia*. Herring had significantly lower relative swimming speeds (vertical and horizontal) than sprat when both species were preying on *Artemia*, which indicates that herring prefers to ingest particles in closer proximity to their eyes than sprat. Transferred into energy costs associated with feeding, the present results showed that herring had a clear energetic advantage above sprat. This can be seen as possible explanation for higher growh rates of juvenile herring compared to sprat. Vertical swimming speeds (body length s^{-1}) had a great impact on the total swimming speed at concentrations >20 l^{-1}, but were less

pronounced at lower prey concentrations. At high prey concentrations vertical swimming speeds were up to 1.6-times higher than horizontal speeds. Transferred into energy costs this indicates that the high vertical speeds are energetically efficient only at prey concentrations above ~14 l^{-1}, which is in accordance with the observed behaviour of sprat and herring showing almost no vertical swimming at lower prey concentrations (< 15 l^{-1}).

The knowledge and data obtained by the present study can serve as basis for the development of bioenergetics budgets for sprat considering (I) body mass and temperature effects on metabolic rates, (II) the influence of activity on metabolism in spontaneously active sprat, (III) feeding rates in relation to prey concentrations and types and (IV) foraging related swimming patterns in relation to prey concentrations.

Zusammenfassung

Die europäische Sprotte (*Sprattus sprattus* L.) ist ein kleiner pelagischer Clupeide mit einer weiten Verbreitung in den Schelfregionen des Nordostatlantiks. Besonders in der Ostsee gelten sie als Schlüsselart, da sie auf der einen Seite als Hauptnahrung für den Dorsch dienen und auf der anderen Seite eine der häufigsten planktivoren Fischarten darstellen. Bisherige Quantifizierungen der top-down Kontrolle der großen Sprottbestände müssen jedoch aufgrund der mangelnden Datenlage zur Tagesration von Sprotten als unzureichend betrachtet werden. Das grundlegende Ziel der vorliegenden Arbeit war es physiologische Prozesse und Verhaltensmuster zu untersuchen, welche für die laufende Entwicklung von bioenergetischen Wachstumsmodellen und Populationsmodellen für Ostseesprotten von Relevanz sind.

Als zentrale Komponente eines bioenergetischen Modells für Sprotten wurde der Einfluss von Temperatur und Körpergröße auf Stoffwechselraten bestimmt (**Manuscript 1**). Dafür wurde der Sauerstoffverbrauch (M_{O2}; mgO_2 $Fisch^{-1}h^{-1}$) von Sprotten (3.1-9.7 g Nassgewicht) in einem *intermittent-flow* Respirometer bei neun verschiedenen Temperaturen zwischen 9 und 21°C gemessen. Der Standardstoffwechsel (R_S), berechnet als 10% Perzentil wiederholter Messungen, war dabei im Mittel nur 12% niedriger als der Routinestoffwechsel (R_R) und immer noch von andauernden Schwimmaktivitäten der Fische beeinflusst. Die Stoffwechselraten stiegen exponentiell mit der Temperatur (Q_{10} = 2.2) in ähnlicher Weise wie es bereits für andere Clupeoide bekannt ist (Q_{10} = 1.8-2.2). Allerdings ergab sich ein höher Exponent für die Größenabhängigkeit des Stoffwechsels (b = 1.073) als der typischer Weise verwendete Exponent von b ~ 0.8. Dies ist höchst wahrscheinlich durch die permanente Schwimmaktivität der Sprotten begründet. Die hohen andauernden Schwimmaktivitäten und der geringe Unterschied zwischen Standard-und Routinestoffwechsel weisen darauf hin, dass das theoretische Konzept einer Standardstoffwechselrate unter Ausschluss jeglicher Spontanaktivität für pelagische Schwarmfische nicht sinnvoll ist. Da das Schwimmen generell einen großen Einfluss auf die Stoffwechselrate bei Fischen hat, wurden die energetischen Kosten spontaner Schwimmaktivitäten anhand einiger Respirometrie-Experimente bestimmt, bei denen eine zeitgleiche Bildaufnahme der Fische vorlag (**Manuscript 2**). Dafür wurden nur Messungen aus der Akklimatisierungsphase der Versuche (kurz nach Einsetzen der Tiere ins Respirometer) verwendet, da hier die Schwimmaktivitäten und Sauerstoffverbrauchsraten variabler waren als in den späteren Routinephasen. Ein automatisches Bildanalyseprogramm zur Fischverfolgung wurde verwendet, um anhand der zeitgleich aufgenommenen Bilder verschiedene Bewegungsmuster wie die mittlere Schwimmgeschwindigkeit und die Rate der Körperdrehungen zu bestimmen. Die Spontanaktivität war dominiert durch niedrige Schwimmgeschwindigkeiten (~ 0.57 Körperlängen s^{-1}) und häufige, scharfe Körper-drehungen (>90°). Diese Bewegungsmuster konnten durch ein nichtlineares Modell mit dem mittleren Sauerstoffverbrauch in Beziehung gesetzt werden. Die Temperatur (10-19°C) hatte

keinen direkten Einfluss auf die Schwimmgeschwindigkeiten oder die Anzahl der Körperdrehungen>90°, jedoch stieg der Sauerstoffverbrauch bei höheren Temperaturen steiler mit der Schwimmgeschwindigkeit an als bei niedrigeren Temperaturen. Das finale Modell für die energetischen Kosten spontaner Aktivität beinhaltet mittlere horizontale Schwimmgeschwindigkeiten sowie die Anzahl der Drehungen>90° und kann angewendet werden um die Schwimmkosten im Feld abzuschätzen, wenn die Häufigkeit dieser Bewegungsmuster bekannt ist.

Bislang liegen keine Informationen zur Abhängigkeit der Fressrate von der Beutekonzentration bei Sprotten vor, obwohl diese Information essentiell zur Beurteilung der maximalen Nahrungsaufnahme ist. Sprotten und Heringe (*Clupea harengus* L.) konkurrieren um dieselben Nahrungsressourcen, vor allem während der juvenilen Lebensphase in der beide Arten in gemischten Schwärmen in den flachen Küstenregionen vorkommen. Bislang gibt es aber keine vergleichende Arbeit zur partikulären Fressrate dieser beiden Fischarten. Um die Abhängigkeit der Fresserate von der Beutekonzentration (1-60 l^{-1}) bei juvenilen Sprotten und Heringen zu bestimmen wurden daher umfangreiche Laborversuche durchgeführt (**Manuscript 3**). Dabei wurden zwei verschiedene Beutearten verwendet, welche sich durch ihr Fluchtverhalten in Anwesenheit eines Räubers unterscheiden. Es wurde angenommen, dass das Fressen von nicht flüchtenden *Artemia salina* Nauplien die maximal mögliche Fressrate widerspiegelt, vergleichbar zum Fressen von Cladoceren in der natürlichen Ernährung, während Experimente mit *Acartia tonsa* (Copepoda: Calanoida) die Bestimmung der Fressrate bei einer natürlichen Beuteart mit starken Fluchtreflexen ermöglichten. Während Heringe bei Konzentrationen über 50 l^{-1} gelegentlich zum Filtrieren übergingen, blieben Sprotten strikt bei der partikulären Nahrungsaufnahme. So konnte erstmals systematisch gezeigt werden, dass Sprotten nur zum Fressen einzelner Partikel durch Schnappbewegungen fähig sind und im Gegensatz zu Heringen nicht die Möglichkeit haben bei hohen Beutedichten zum Filtrieren überzugehen. Beide Fischarten zeigten eine *type-II-functional response* (asymptotisch steigende Aufnahmerate mit der Beutedichte) mit signifikant niedrigeren Fressraten bei Versuchen mit *A. tonsa* im Vergleich zu Experimenten mit Artemien. Beim Fressen von Copepoden zeigten beide Fischarten eine S-förmige Körperverbiegung unmittelbar vor dem Schnappen, was durch das Fluchtverhalten von *A.tonsa* verursacht wurde. Die Parameter der *functional response*-Kurven waren nicht signifikant verschieden zwischen Sprotten und Heringen, außer bei Konzentrationen unter ~ 40 Copepoden l^{-1} wo Heringe höhere Schnappraten erreichen konnten. Die Ergebnisse dieser Studie erweitern das Verständnis von Interaktionen zwischen Planktonpopulationen und konkurrierenden planktivoren Fischarten. Die Parameter der *functional response*-Kurven können bioenergetische Modelle verbessern, welche in umfassende Ökosystemmodelle eingebunden sind. Da aber eine realistische Modelstruktur auch Abschätzungen der Schwimmaktivitäten bei verschiedenen Beutedichten enthalten muss, wurden die Fress-Experimente auch dazu verwendet die fressbedingten Bewegungsmuster bei Sprotten und Heringen zu untersuchen (**Manuscript 4**). Dafür wurden die Schwimmbewegungen und das Verhaltensmuster anhand digitaler Bilder bestimmt,

wobei eine Kamera über dem Becken Bilder zur Analyse der horizontalen Bewegungsmuster lieferte, während eine Unterwasserkamera Bilder zur Untersuchung vertikaler Bewegungen ermöglichte. Beide Fischarten steigerten die horizontale Schwimmgeschwindigkeit (cm s^{-1}, BL s^{-1}), die mittlere Körperdrehungsrate (° s^{-1}), die Anzahl starker Körperdrehungen (<90°; $Fisch^{-1}h^{-1}$) und die Varianz der Beschleunigung (cm s^{-2}) mit der Beutekonzentration. Für diese Bewegungsmuster konnten nichtlineare (Gompertz-Funktion) Mixed-Effects Modelle angepasst werden, welche die Unterschiede zwischen den Beutearten und den beiden Fischarten berücksichtigen, sowie das widerholte Messen an der gleichen Fischgruppe mit einbeziehen. Insgesamt ergaben sich für alle getesteten horizontalen Schwimm-Muster signifikant niedrigere Maximalwerte wenn die Fische Copepoden gefressen haben anstelle von Artemien. Heringe zeigten insgesamt niedrigere relative Schwimmgeschwindigkeiten (horizontal und vertikal) als Sprotten wenn beide Arten Artemien gefressen haben. Dies deutet darauf hin, dass Heringe bevorzugt Beute nahe dem Gesichtsfeld schnappten. Übertragen in Energiekosten durch das Fressen deuten die Ergebnisse dieser Studie darauf hin, dass Heringe einen klaren energetischen Vorteil gegenüber Sprotten haben. Dies wird als mögliche Erklärung für die höheren Wachstumsraten juveniler Heringe gegenüber Sprotten angesehen. Die vertikalen Schwimmgeschwindigkeiten hatten einen großen Einfluss auf die Gesamtschwimmgeschwindigkeit bei Konzentrationen >20 l^{-1}, waren aber weniger stark ausgeprägt bei niedrigeren Konzentrationen. Bei hohen Beutekonzentrationen (>40 l^{-1}) waren die vertikalen Schwimmgeschwindigkeiten bis zu 1.6-mal höher als die horizontalen Geschwindigkeiten. In Energiekosten übertragen würde dies bedeuten, dass sich die hohen vertikalen Geschwindigkeiten erst ab einer Beutekonzentration von etwa 14 l^{-1} lohnen. Dies stimmt mit dem beobachteten Verhalten von Sprotten und Heringen überein, welche bei niedrigen Beutekonzentrationen (<15 l^{-1}) kaum vertikale Bewegungen aufzeigten.

Die Ergebnisse der vorliegenden Arbeit können als Basis für die Weiterentwicklung eines bioenergetischen Modells für Sprotten dienen und liefern Informationen zum (I) Einfluss von Körpergröße und Temperatur auf Stoffwechselraten, (II) zu den energetischen Kosten spontaner Schwimmbewegungen, (III) Informationen zur Fressrate in Abhängigkeit von Beutekonzentration und -art bei Sprotten und Heringen gleicher Größe, sowie (IV) Informationen zur Abhängigkeit verschiedener Bewegungsmuster von Beutekonzentration und –art.

1 General introduction

1.1 Sprat and its ecological role

European sprat (*Sprattus sprattus* L.) is a small clupeid fish with a wide distribution covering the North and Baltic Seas, the Irish Sea, Bay of Biscay down to Morocco, the northern parts of the Mediterranean, the Black and Adriatic Seas (Whitehead 1985; Muus and Nielsen 1999). Sprat is a marine pelagic and usually inshore fish, which tolerates salinities down to 4 PSU and sometimes migrate into estuaries (Whitehead 1985). It is regarded as a classical r-strategist (McArthur and Wilson 1967; Roff 1992) with a short live span, maturing early (age-1 or 2; ICES 2002) and producing up to 10 egg batches throughout the spawning season (George and Alheit 1987). It rarely reaches ages >5 years (Bailey 1980) or a length >16 cm (Whitehead 1985). Among European waters different sub-populations with genetic differences were reported (Debes *et al.* 2008; Limborg *et al.* 2009) and sub-species have been recognized. Sprat coexists with other clupeoids in the same habitat, *e.g.* with European anchovy (*Engraulis encrasicolus*), European sardine (*Sardinops sagax*) or Atlantic herring (*Clupea harengus*). The ecological importance of these species is mainly caused by the fact that they can significantly structure an ecosystem as they feed on a multitude of plankton and transfer energy to larger piscivorous species (Cury *et al.* 2000). This intermediate trophic level is often occupied by one or two small pelagic fish species in a system, *e.g.* by sardine and anchovy in upwelling regions (Cury *et al.* 2000; van der Lingen *et al.* 2009) or by sprat and herring in the Baltic Sea as dominant planktivorous fish species (Möllmann *et al.* 2004). In the Baltic Sea sprat are a major prey source for cod and other piscivores like salmon (*Salmo salar* L.), but they also prey on cod eggs (Köster and Möllmann 2000) which is called a prey-to-predator loop (Möllmann *et al.* 2008). Changes in hydrography and human exploitation caused a shift from a cod-dominated system in the 1980s to a clupeid (or sprat-) dominated system in the early-1990s (Casini *et al.* 2008; Möllmann *et al.* 2009). The increase of the sprat stock in the Baltic Sea had consequences for both sprat and herring as the weight-at-age (WAA) and body condition declined in the last two decades in both species (Cardinale and Arrhenius 2000; Cardinale *et al.* 2002; Casini *et al.* 2011). This decrease is partly caused by an enhanced intra-and interspecific competition for food resources (Möllmann *et al.* 2005; Casini *et al.* 2006) and a change in the zooplankton taxonomic composition. In the northern areas of the Baltic proper the drop in condition of sprat and herring was stronger than in the more southern parts as the sprat population increased the most in the northern Baltic Sea regions (Casini *et al.* 2011). Recently, the cod stock increased in the central Baltic Sea (ICES-subdivision 25; around the Isle of Bornholm) which will have a strong effect of sprat in this area, but very limited effect on the whole Baltic sprat stock which is currently largely out of reach for cod (ICES 2012). Beside its high ecological importance, sprat also represents the most abundant commercially exploited fish in the Baltic Sea with total landings of 268 kt in 2011 (ICES 2012).

1.2 Feeding ecology of sprat and herring

Sprat and herring are zooplanktivorous fishes which feed mainly on adults and late copepodites of *Temora longicornis*, *Acartia* spp. and *Pseudocalanus elongatus* as well as on the cladocerans *Bosmina maritima* and *Bosmina longispina* depending on season, fish body size (Casini *et al.* 2004; Bernreuther 2007), geographical region (Lankov *et al.* 2010) and local changes in the zooplankton community. Both clupeids consume a large amount of the annual zooplankton production in the Baltic Sea (60-80%; Arrhenius and Hansson 1993) and have a distinct impact on the zooplankton community by feeding selectively on larger, reproducing individuals (Flinkmann *et al.* 1998). However, herring also preys on smaller copepodite stages (Möllmann *et al.* 2004), mysids and fish larvae (Blaxter 1990; Arrhenius and Hansson 1993; Bernreuther 2007) and sprat are known to prey to a large extent on pelagic fish eggs, *e.g.* from cod in the Baltic Sea (Köster and Möllmann 2000; Möllmann *et al.* 2008) or plaice in the North Sea (Plirú *et al.* 2012).

Most clupeoid fishes, such as anchovies, sardines or herring can switch between particulate and filter feeding (*e.g.* Leong and O´Connell 1969; Gibson and Ezzi 1985; van der Lingen 2006). The ability to switch between these feeding modes makes these species highly opportunistic foragers which are able to maximize their energy intake through employing the feeding mode most appropriate to a particular food environment (van der Lingen *et al.* 2009). Sprat are, however, assumed to be obligate particulate feeder (Bernreuter 2007; Peck *et al.* 2012) which results in a higher dependence of growth performance on the abundance of optimal (larger) prey sizes compared to fish which are able to switch to filter-feeding when only smaller prey is available. During particulate feeding a fish´s feeding rate is relatively fixed by the handling time (opening and closing of the mouth) while swimming through a zooplankton patch (Peck *et al.* 2012). This has direct implications for the outcome of intra- and inter-specific competitive behaviour between sprat and herring and probably explains why sprat is such a small bodied fish as they are unable to filter-feed. Filter feeding seems to be associated with body size as it occurs mainly in fish with a larger maximum size (*e.g*. Atlantic herring ~ 28 cm; Atlantic menhaden ~ 50 cm; Fishbase 2009) than sprat (max 10-13 cm; Fishbase 2009) or is energetically more efficient in larger (*e.g* sardine) compared to smaller species (*e.g.* anchovy; van der Lingen 2006). Juvenile Atlantic herring, however, also feed mainly by biting (Gibson and Ezzi 1985) and partly prey on the same zooplankton species and sizes as sprat (Maes and Ollivier 2002). This makes direct comparisons of the feeding behaviour between juvenile sprat and herring an important aspect for understanding intra-specific competition and top-down impacts in shallow-coastal regions by these two species.

Besides intraspecific food competition, schooling fish also have the particular problem that other school members are potential food competitors. Schooling fish may therefore reduce competition for food when resources are limited by increasing inter-fish distances and by segregating into groups of similar body size (Robinson 1995). Some species may also be able to reduce their prey handling time when the school size increases (Street *et*

al. 1984). For sprat it has been proposed that they can get food-limited during the day when they swim in dense schools (Hawkins *et al.* 2012) and that such schools can deplete local zooplankton patches rapidly (Maes *et al.* 2005), but so far no laboratory experiments have been conducted to determine feeding rates of sprat in relation to prey concentrations (*i.e.* functional response).

1.2.1 Functional response

Functional response curves predict the feeding rate of an animal as a function of prey density and are applied in models that predict foraging behaviour and growth rates of planktivorous fishes in freshwater (Stockwell and Johnson 1997; 1999) and marine systems (Moss and Beauchamp 2007). Functional response basically takes three forms (Holling 1959; 1966; Figure 1-1): The type I functional response models the increasing consumption linearly with increasing prey density and is mainly observed in filter feeding organisms foraging in homogenous prey concentrations. Type II functional response characterizes consumption rate as decelerating asymptotically with prey density. Then, prey handling time becomes a factor that limits the consumption rate at high prey concentrations. The type III functional response takes a sigmoid form and assumes that predators are inefficient in finding prey at lower prey denstities or that there is a threshold level below the predator does not respond. In bioenergetics models for clupeoid fishes, type II functional response equations are applied for multiple prey types (*e.g.* Megrey *et al.* 2007; Politikos *et al.* 2011) considering prey switching behaviour of the predator. However, prey handling times may be different for prey types with different escape abilities, but this is not considered in such models. Furthermore, no laboratory studies have been conducted to identify the type of functional response in sprat or herring during particulate feeding at low prey concentrations. However, in the field prey is patchy distributed on almost any temporal and regional scale and typically found concentrations can range from less than 10 l^{-1} (Colebrook 1979; Broekhuizen and McKenzie 1995) to more than 1000 l^{-1} in areas of high aggregation (Folt and Burns 1999). Previous investigations on feeding behaviour of zooplanktivorous fish were often focused on factors triggering filter-feeding, such as light conditions (Batty *et al.* 1990; Macy *et al.* 1998) or prey concentrations and types (Gibson and Ezzi 1990; Busky 1994; van der Lingen 1994; Garrido *et al.* 2007). Although in these studies particulate feeding was observed and related to prey concentrations, the type of functional response was not investigated.

1.2.2 Foraging-related swimming activities

Besides information on functional response also swimming speeds of planktivorous fish during feeding should be included in bioenergetics models. This will allow considering effects of different prey concentrations on the overall energy demand and therefore the net available energy. Hence, modelling activity as a function of prey density will improve fits between observed and predicted food consumption rates (Chipps and Wahl 2008). In a first

step one needs to quantify the swimming activities of fish in relation to various different prey concentrations. In order to measure swimming activity in feeding fish one can either use hydroacoustic methods for field measurements (*in situ*) or determine swimming patterns in laboratory experiments. For anchovies prolonged speeds between 2 and 9 BL s^{-1} were measured *in situ* (Taylor *et al.* 2007) and although such speeds are likely to occur during swimming and foraging, *in situ* measurements provide no detailed information about the prey concentrations in which the fish were feeding. Therefore, laboratory experiments are more suitable for the determination of activity rates (including different swimming patterns) in relation to various prey concentrations than observations made in the natural environment. Previous laboratory studies on clupeoids revealed that swimming speeds during particulate feeding increase with increasing prey concentrations (*Sardinops sagax*; van der Lingen 2006) and can be 2-3 times higher than during non-feeding (*Engraulis capensis*, James and Probyn 1989). However, so far swimming speeds or other feeding related movements were not tested as functions of prey concentrations for sprat or herring. In bioenergetics models for other clupeoids swimming speeds are, to the best of my knowledge, not directly modelled as function of prey densities due to a lack of data. Either a constant cruising speed is assumed (*e.g.* 2 BL s^{-1} for herring; Varpe and Fiksen 2010) or swimming speeds vary only with feeding modes and prey sizes (Politikos *et al.* 2011). For the realistic evaluation of energy costs while foraging, one needs to investigate the relationship between swimming patterns and prey concentrations like discussed in several publications on optimal foraging theories (*e.g.* Charnov 1976; Ware 1978). Optimal foraging theories postulate that a predator adjusts its swimming behaviour in relation to prey density in order to maximize the net energy intake. Thus, detailed information on swimming patterns of sprat and herring while feeding on a range of prey concentrations can improve bioenergetics based estimates on total energy costs of feeding and hence the net energy intake rates in relation to prey density.

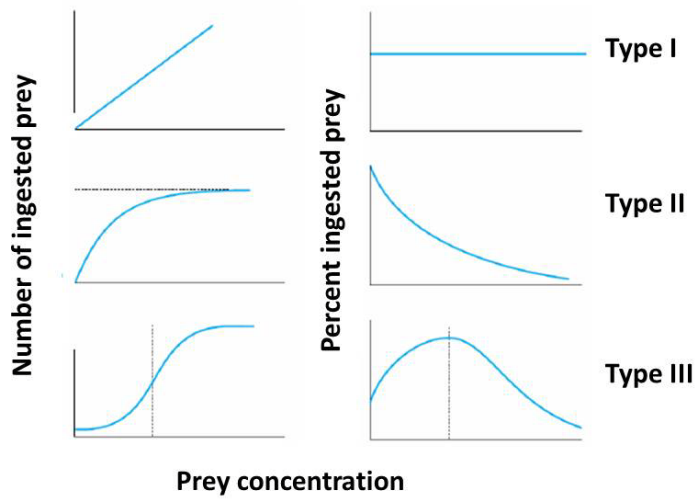

Figure 1-1 Schematic of the three types of functional response after Holling (1959; 1966). Type I is typical for filter-feeding organisms, Type II is used for particulate feeding planktivorous fishes and Type III assumes that the predator is inefficient at finding prey at lower prey concentrations

1.3 Fish bioenergetics

The quantitative assessment of the predation impact of pelagic fish on zooplankton requires reliable data on the daily ration of the fish. One way of predicting daily ration of fish is through the use of bioenergetics models, which balance the consumed energy over metabolic processes and growth. Such bioenergetic models for fish are commonly applied in ecosystem models and have been used with increasing frequency (Hartmann and Hayward 2007; Chipps and Wahl 2008). The consumed energy (C) for an individual fish over a defined time period can be described by the following mass balance equation (Winberg 1960):

$$C = (G_S + G_R) + (M_S + M_A + SDA) + E + U \quad (1)$$

Thereby G is the energy used for growth processes and is divided into somatic growth (G_S) and reproductive or gonadal growth (G_R), M_S is the energy loss due to standard metabolism and M_A is the metabolic loss for swimming activity, SDA (specific dynamic action) describes the metabolic increase after feeding (digesting and assimilating food) above maintenance costs, E is the energy lost by egestion and U is the energy loss by excretory products, particularly nitrogenous products such as ammonia and urea. All components of this energy budget need to be expressed in the same units, which are generally wet or dry mass for biomasses and Joules or calories for energy equivalents. Carbon and nitrogen products are typically expressed in amount of gained or lost energy. The reliability of growth and consumption predictions made from bioenergetics models needs to be tested among a broad range of body masses, temperatures and ration levels (Chipps and Wahl 2008). The applied model should be based on parameter estimates derived from the target species or at least from closely related species.

Another approach to determine the daily ration (or consumption) in fish requires measurements of gut contents over a diel cycle coupled with estimates of gastric evacuation rates (Elliott and Persson 1978; Eggers 1979; Pennington 1985). For both sprat and juvenile herring gastric evacuation rates have been determined in laboratory experiments and the results were combined with field stomach samples (Bernreuther *et al.* 2008; 2009). However, different modelling approaches may result in different estimations of daily ration. For instance, Maes *et al.* (2005) reported for juvenile sprat and herring that a bioenergetics model resulted in overestimated daily rations, whereas the evacuation model underestimated the daily ration. Bernreuther (2007) also reported such differences between an evacuation model and energy demands calculated by a bioenergetics model for sprat. Thereby the evacuation model resulted in exceptionally low daily rations which were not sufficient to cover energy demands for maintenance metabolism in sprat. The author partly explained this by the fact that stomach samples were taken during summer in the Bornholm Basin, which was obviously no feeding habitat for sprat in summer months. However, bioenergetics models applied for sprat were so far lacking on species specific input parameters such as for temperature and mass related metabolic rates. Previous models for

sprat were either based on sub-models including mainly metabolic parameter estimates borrowed from other species (*e.g.* Arrhenius 1998a; Bernreuther 2007) or were focused on larval stages only (*e.g.* Daewel *et al*. 2008). Recently, Frisk (2012) applied a simple bioenergetics model for sprat partly using results from the present investigation (presented in Manuscript 1), but also parameter estimates borrowed from other species. The first models for Atlantic herring were developed by Rudstam (1988) and later by Arrhenius and Hansson (1993) for sprat and herring. However, these models were also mainly based on parameter estimates derived for other species (Bernreuther 2007). More recent models for clupeoids are often based on the NEMURO model family. The PICES MODEL Task Team constructed a lower-trophic ecosystem model called NEMURO (North Pacific Ecosystem Model for Understanding Regional Oceanography) in order to study marine ecosystems (Kishi *et al.* 2007). This model was later extended by a fish growth bioenergetics model (NEMURO.FISH) designed to use as input the plankton densities generated by the NEMURO model (Kishi *et al.* 2011). The fish bioenergetics model was also applied to model growth in Pacific herring (*C. palasii*, Mergrey *et al.* 2007) and anchovy (*E. encrasicolus*, Politikos *et al.* 2011). However, Megrey *et al.* (2007) still use many parameter estimates reported by Rudstam (1988), which were not obtained from experiments with herring. The bioenergetics component of the NEMURO.FISH model is basically an implementation of the Wisconsin style model (Hewett and Johnson 1992; Hanson *et al.* 1997), but differs in the way how maximum daily consumption is determined (Kishi *et al.* 2011). Within the NEMURO.FISH model the iterative calibration of the *p*-value (representing prey availability as fraction of maximum consumption) is replaced by a type-II multispecies functional response, which uses prey densities to then determine daily consumption (Kishi *et al.* 2011). Consequently, knowledge on species-specific functional response parameters in relation to different prey concentrations and types is required. Furthermore, information of swimming patterns associated with feeding should be included so that energy requirements for foraging can be estimated.

1.3.1 Metabolic rates

Fish metabolic rates are usually measured as oxygen consumption rates (Chech 1990) at different water temperatures, body masses, feeding levels or swimming speeds. Alternatively, metabolic rates of clupeids have been estimated from energy losses during starvation (*e.g.* Logerwell 2001; Bernreuther 2007), but since oxygen consumption rates can be related to more different activity levels and feeding states, this became the standard approach for determining metabolic rates in fish.

Metabolic rates of unfed fishes were classically divided into standard (R_S), routine (R_R) and active metabolism (R_A) depending on the activity level of fish during measurements (Fry 1971). **Standard metabolism (R_S)** is an approximation of a minimum metabolic rate and was first defined by Krogh (1914) as the "nearest attainable approximation to the basal metabolism which one would obtain when all organs are at rest". Further classifications

defined R_S as the metabolism of a resting animal in a post-absorptive state, acclimated to experimental conditions (Fry 1971; Brett and Groves 1979). By definition, this metabolic rate excludes any kind of internal or external work, even digestion and all movements, except the ventilation of the gills for respiration (Zimmermann and Kunzmann 2001). The energy used during R_S is basically used for processes such as the maintenance of the mitochondrial H^+ gradient, protein turnover, repair or turnover of cellular structures, active transports of solutes, blood circulation and ventilation (Rolfe and Brown 1997; Hulbert and Else 2000). For measuring R_S in fish one needs to exclude food consumption, stress, excitement and spontaneous activities. Avoiding digestion processes is easy as fish could be fasted for several days (depending on the species and diet) without direct starvation impacts. Long enough acclimation periods will ensure that fish are fully acclimated to experimental conditions and therefore neither stressed nor excited during experimentation. However, the complete exclusion of spontaneous activities is difficult to ensure in all pelagic fishes and does not seem to be meaningful for all species. Therefore Fry (1971) suggested determining standard metabolism as the value found at zero activity by relating metabolic rate to random physical activity in fish in the post-absorptive state. However, extrapolating to zero swimming speed is not always reliable as fish may get excited at low swimming speeds (Brett 1965; Bushnell *et al.* 1994) and different mathematical models can yield in different extrapolations to zero. Using an exponential function will give more reasonable estimates for a standard metabolism when extrapolated to zero swimming speed than the application of a power function (Korsmeyer *et al.* 2002). On the other hand is a power function derived from hydrodynamic principles (Papadopoulos 2008) and was recommended for comparisons among species when standard metabolic rates are variable (Korsmeyer *et al.* 2002). Thus, the use of a power function of the form $MO_2 = a\ U^{\ c}$ (with MO_2 = oxygen consumption; U= swimming speed) was favoured in recent investigations on metabolic costs for swimming in fish (*e.g.* Videler 1993, Ohlberger *et al.* 2006; 2007; Steinhausen *et al.* 2010) allowing direct comparisons among species. Another approach to approximate for standard metabolic rates was proposed by Blaxter (1989) where the lowest values from repeated observations are defined as R_S. Hence, in the past decades several mathematical approaches using percentiles (*e.g.* Steffensen *et al.* 1994; Hermann and Enders 2000) or different quantiles (*e.g.* Couturier *et al.* 2007; Dupont-Prinet *et al.* 2010) from frequency distributions were applied in order to determine R_S from long term measurements (several hours to days) when low values occurred frequently. This does not guarantee that the fish showed no activity during experimentation, but the risk that the same level of activity was observed regularly over a long study period is relatively small. However, there is still no generally accepted method to quantify R_S from such observations for pelagic fishes and R_S has not been determined for sprat so far. In contrast to standard metabolism, **routine metabolic rates (R_R)** include by definition costs for spontaneous activities (Fry 1957) and are therefore much easier to measure in pelagic fishes. However, without simultaneous measurements of swimming activities, this metabolic level is not clearly defined and therefore not suitable for comparisons among species or groups and not very useful in bioenergetics modelling.

The **active metabolic rate (R_A)** is the maximum rate of aerobic metabolism, inducible by swimming and was classically defined as metabolism during maximum sustainable speeds (Fry 1971; Brett and Groves 1979). Such metabolic rates were usually measured for individual fish swimming against a constant current in a tunnel respirometer (Blazka *et al.* 1960; Gehrke *et al.* 1990; Dickson *et al.* 2012). It was commonly assumed that active metabolism ranges from one to three times standard metabolism and therefore a simple activity multiplier was used in bioenergetics models to account for the energy costs for swimming above maintenance costs (Winberg 1960; Kitchell *et al.* 1977; Hewett and Johnson 1992). However, the swimming speed at which R_A will occur depends on the swimming mode and life style of a species and activity costs may be larger or more variable (Koch and Wieser 1983; Boisclair and Legett 1989; Tang *et al.* 2000). An individual fish may use different swimming modes, *e.g.* during searching for food the swimming mode can be defined as *unsteady*. This unsteady swimming is characterized by spontaneous activities, such as low to moderate speeds and frequent changes in speed and direction (Boisclair and Tang 1993; Videler 1993; Tang *et al.* 2000). In contrast to this, *steady* swimming may be performed during migratory activity where the fish swims linearly at moderate to high speeds. During unsteady swimming, R_A will be reached at relatively low swimming speeds compared to steady swimming as spontaneous activity includes expensive manoeuvres in terms of energy costs, such as changes in speed and direction (Webb 1991; Enders and Herrmann 2003; Steinhausen *et al.* 2010). Many fishes may also use a third swimming mode during prolonged swimming where the fish accelerates rapidly and coasts while losing speed. This *burst-and-coast swimming* is frequently shown by clupeid fishes (Blake 1983; Videler 1993) and allows saving energy compared to continous steady swimming at the same speed (Videler and Weihs 1982).

In some cases metabolism can be higher than R_A, if for example fish were forced to swim at their maximum possible speeds for a limited period of time or fish were exhausted after chasing. This metabolism is then termed **maximum metabolic rate** (MMR) and gives an indication of how a fish can cope with various stressing conditions, like temperatures below or above the temperature optimum, hypoxia or increased pH and CO_2. The difference between standard metabolism and maximum metabolic rate is called the *aerobic scope* or *scope for activity*. A reduction in aerobic scope limits the energy available for activity, growth, reproduction and other vital rates. However, due to the high sensitivity of clupeoids in tanks, studies on maximum metabolic rates were not conducted so far.

1.3.2 Specific dynamic action (SDA)

The most accepted working definition of **SDA** is the accumulated energy induced by the ingestion, digestion, absorption, and assimiliation of a meal (Jobling 1994). The term SDA was originally introduced by Max Rubner (1902) as “spezifisch dynamische Wirkung”, but later this has also been termed the heat increment of feeding, dietary thermogenesis or thermic effect of feeding (Secor 2009). By definition SDA excludes the cost of activity

involved in acquiring and ingesting the meal, as well as any spontaneous activity that takes place during digestion (Secor 2009). In fish SDA increases as a function of meal energy (Secor 2009) and is equivalent to 9-20% of the total ingested energy (Miura *et al.* 1976, Xie *et al.* 1997; Owen 2001). SDA in bioenergetics models is often considered to be a constant fraction of assimilated energy by: SDA = a_{SDA} (C-E), where a_{SDA} is the SDA coefficient, C is the consumption rate and E is the egestion rate. However, most studies on SDA effects were conducted on piscivorous fish important to commercial or recreational fishing, or those used in aquaculture (Secor 2009). In bioenergetics models for clupeoids SDA is assumed as 10-17.5% of the assimilated energy (Kiørboe *et al.* 1987; Rudstam 1988; Arrhenius 1998b) depending on ontogenetic stage (Megrey *et al.* 2007). For juvenile young-of-the-year sprat SDA was determined to be ~ 10% of the ingested energy (Herrmann *et al.* 2004). The particular problem in measuring SDA in zooplanktivorous fishes is that swimming activities during foraging and digesting cannot be avoided. However, there are basically two possibilities to solve this problem: 1) by removing the costs for activity with mathematical approaches, *e.g.* by quantile regressions (Chabot and Claireaux 2008) or 2) by measuring the overall metabolic costs during feeding as *feeding metabolism* (Jobling 1994) instead of trying to measure SDA effects solely. Together with information on energy costs for feeding related swimming patterns (foraging costs), the latter method allows indirectly to approximate for SDA by subtracting foraging costs from the total feeding metabolism. However, there is no information on feeding related swimming patterns, costs for swimming or SDA coefficients for adult sprat.

1.3.3 Egestion and excretion

Egestion (E) is generally modelled as a constant proportion of consumption and was assumed to be 16% for adult herring (Rudstam 1988; Arrhenius 1998b; Megrey *et al.* 2007). For herring larvae values between 10 and 17.5% were reported (Kiørboe *et al.* 1987; Klumpp and von Westernhagen 1987; Limburg 1994) and consequently Megrey *et al.* 2007 used 12.5% for age-0 herring in their model. A similar value of 12.6% was applied by Politikos *et al.* (2011) in a bioenergetics model for anchovy. Excretion (U) is assumed to be a constant fraction of assimilated energy and was assumed to be 10% in bioenergetics models for herring (Rudstam 1988; Megerey *et al.* 2007) based on rates reported by Elliott (1976) for brown trout. For younger herring (age-0) lower values were reported and Megrey *et al.* (2007) used 7.5% in their model for age-0 herring. The amount of energy lost by E and U can be measured in growth-feeding experiments where fish are fed certain food rations over a defined time period. The net growth efficiency (NGE) measures the conversion efficiency of the food consumed in excess of the maintenance requirements (Wootton 1998). NGE integrates the energy lost in faeces and nitrogenous wastes and costs for SDA, which depend on the prey type (or food quality).

1.3.4 Temperature and body mass effects on metabolic rates

Fish are in general dependent on several external (ecological) and internal factors, which control or synchronize activities or functions, including metabolic rates and behaviour. In the following passage I will refer to the most important and presently investigated effects affecting fish metabolic rates, namely temperature and body mass.

Temperature is the most important determining environmental factor affecting behaviour and metabolic processes of fish that influence predator-prey interactions (Wootton 1998). Changes in local temperatures will cause changes in physiological rates and therefore directly affect the growth potential for a fish. Metabolic rates of fishes are usually modelled as exponential function of temperature (Fry 1971; Brett and Groves 1979), resulting mostly in Q_{10} values ~ 2 (Clarke and Johnston 1999). Although temperature effects on metabolic rates at different activity levels were widely investigated, only a few studies considered the direct influence of temperature on routine swimming speed (Ottmar and Hurst 2012). Generally, theories and observations support the existence of three different patterns (Ottmar and Hurst 2012): 1) an increase in swimming speed with an increase in temperature parallel to the physiological response, 2) an increase in swimming speed with a deviation from the preferred temperature or 3) maintenance of constant speed across temperature (Precht 1958; Fraenkel and Grunn 1961; Bennett 1990). For adult clupeids it was assumed that swimming speeds remain constant at temperatures above a species specific threshold and decrease with decreasing temperatures below this value, *e.g.* below 9°C in herring (Rudstam 1988) or alewife (Stewart and Binkowski 1986) and below 12°C in anchovy (Politikos *et al.* 2011). However, the effect of temperature on swimming speeds has not been investigated in laboratory studies for sprat and herring and it is still unknown how costs for spontaneous or routine swimming depend on temperatures in these fishes. Furthermore, there is hardly any information on how standard and routine metabolic rates of sprat depend on water temperature, so that parameter estimates in bioenergetics models were borrowed from related species (Arrhenius and Hansson 1993; Maes *et al.* 2005).

Body mass is one of the most important intrinsic factors affecting fish metabolic rates. Typically the relationship between metabolic rate (MR) and body mass (M) is expressed by a power function of the form $MR = a M^{b}$ (Ware 1978; Glazier 2005). It has widely been accepted that the scaling exponent is 3/4, the so called“3/4 -power law” after Kleiber (1961). Although interspecific scaling exponents tend to be around 3/4 (Glazier 2005), the intraspecific values can range from 0.4-1.29 (Clarke and Johnston 1999), which emphasizes the necessity to derive species-specific models. Furthermore, metabolic scaling exponents are influenced by other intrinsic or extrinsic factors. Significant effects of temperature on metabolic scaling in fish were reported (Glazier 2005), resulting in lower mass scaling exponents at higher temperatures (*e.g.* Ohlberger *et al.* 2007; 2008). Furthermore, SDA-effects tend to cause *b* to approach 1 (Glazier 2009) and *b*-values were found to differ significantly among species with different life styles: low weight scaling exponents of routine metabolism have been found for less active, demersal species and

higher weight exponents have been found for more active, pelagic fish species (Childress and Somero 1990; Somero and Childress 1990). Likewise, higher exponents were found within a species when measurements were conducted with active fish instead of fish at rest (*e.g.* in pike; Armstrong *et al.* 1992). Exponents referring to early life stages only, tend to be higher than those based on juvenile and adult stages (Riisgård 1998; Glazier 2005). Thus, it is essential to investigate stage specific body mass effects at typical temperatures experienced by the target species.

1.4 Specific goals of the thesis

The present study focuses on physiological and behavioural aspects relevant for the ongoing development of bioenergetics growth and population models for Baltic Sea sprat.

The ***first objective*** was to close identified gaps in knowledge about metabolic rates of juvenile and adult sprat considering the influence of spontaneous swimming patterns, fish body mass and water temperature. Therefore respirometry experiments were conducted with small fish schools at temperatures between 9-21°C and body masses from 3 to 9 g wet mass. A special focus was also on the applicability of methods for distinguishing standard (R_S) and routine (R_R) metabolic rates (**Manuscript 1**) in sprat since there is still no general procedure how to define standard metabolism in permanently active fishes swimming in schools. This investigation forms the basis for bioenergetics based predictions of daily consumption by sprat. During these respirometry experiments video monitoring of fish was conducted and different fish swimming patterns such as mean swimming speeds and sharp turning movements (>90°) were detected by an automated image tracking program specifically developed for this application. It was also tested if measurements from the acclimation phase (beginning of experiments) can be used to determine the effect of spontaneous swimming activities on metabolic rates as during these phases activity and oxygen consumption rates have been shown to be more variable than during the later routine phases which are generally used to determine R_R and R_S in fish. The main goal was to quantify the energy costs for different movement patterns which are assumed to reflect a common behaviour in spontaneously active sprat. Thereby routine metabolic rates were related to spontaneous swimming activities and the influence of temperature on these swimming patterns was investigated (**Manuscript 2**). This study provides important information on energy costs associated with variable, spontaneous swimming patterns in sprat and forms the basis for estimating feeding related energy costs.

The ***second objective*** was to investigate the feeding behaviour of juvenile sprat and herring which form mixed schools in nature and partly prey on the same zooplankton species and sizes. In future bioenergetics models for these species knowledge on functional response parameters and feeding related swimming speeds is required. Therefore extensive laboratory feeding experiments were conducted with juvenile sprat and herring feeding on a range of concentrations of two single prey types with different escape capabilities (non-evasive *Artemia salina* nauplii or highly evasive *Acartia tonsa* copepodites and adults). From

these experiments, functional response curves were developed considering effects of prey type and fish species (**Manuscript 3**). These experiments were also used to investigate feeding related swimming patterns of sprat and herring (**Manuscript 4**). Different components of these swimming patterns were determined, such as accelerations, mean swimming speeds, mean turning rates and numbers of sharp turns (>90°). Horizontal movements were quantified from images of a top-camera, whereas vertical swimming was investigated by the use of underwater images. The results from this study will together with results from Manuscript 2 allow a first approximation of the energy costs associated with feeding in sprat. Furthermore, these results allow to model swimming activities as a function of prey concentrations which can improve predicted food consumption rates in foraging models linked to bioenergetic growth models.

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2 Manuscript 1: Effects of temperature and body mass on metabolic rates of sprat, *Sprattus sprattus* L.

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Abstract

Sprat, *Sprattus sprattus* L., is a small schooling clupeid forming large stocks in several ecosystems. Despite its high trophodynamic impact, little is known about its energy consumption rates. As a central component of a bioenergetic budget metabolic rates of sprat from 3.11 to 9.71 g wet weight (WW) were measured at 9 different temperatures (T) ranging from 9-21°C using a computer controlled intermittent-flow respirometer. Routine metabolism (R_R) was related to T (°C) and WW (g) by R_R=0.074 WW $^{1.077}$ e $^{0.080\ T}$. Standard metabolic rates (R_s) as calculated from the 10% percentiles of the repeated measurements were on average 12% lower and still influenced by continuous swimming activity: R_S=0.069 WW $^{1.073}$ e $^{0.078\ T}$. We interpret the deviation of the scaling exponent *b* from typically found exponents of *b* ~ 0.8 as a consequence of permanently elevated activity level. The high permanent swimming activities also indicated that the concept of standard metabolism may not be meaningful in schooling planktivorous fish. These results suggest that generally in bioenergetic models for clupeid schooling fish the activity multipliers should be chosen very conservatively.

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2.1 Introduction

European sprat (*Sprattus sprattus* L.) like other planktivorous schooling clupeids such as herring (*Clupea harengus* L.), European pilchard (*Sardina pilchardus*) or European anchovy (*Engraulis ringens*) have high predatory impact on plankton communities due their large stock sizes. A well-studied example, Baltic Sea sprat not only influences zooplankton populations (Permanne *et al.* 1994; Arrhenius 1996), but also preys on cod (*Gadus morhua* L.) eggs to an extent that it partly controls the recruitment success of its own predator (Köster and Möllmann 2000). Climate-induced changes in hydrography since the early 1980s have led to a reversal of the dominance of cod and sprat (Köster *et al.* 2001). These changes are viewed as part of regime shift in the Baltic Sea with changes at all trophic levels down to phytoplankton (Möllmann *et al.* 2008). Current quantifications of the top down controls of the large sprat stock, however, have to be regarded as preliminary due to the limited and disputed data on sprat daily rations. Previous estimates on sprat daily rations were based on the gastric evacuation method, but suffer from the fact that experiments were only performed on board of research vessels with trawl caught sprat (Köster *et al.* 1990). Furthermore the stomach samples do not necessarily cover the entire distribution area of the population (Bernreuther 2007). Bioenergetic budgets of sprat used so far were based on parameter estimates derived largely from related or even unrelated species (Arrhenius and Hansson 1993; Arrhenius 1998). To our knowledge, there is no information available for both temperature and body mass effects on metabolic rates of sprat, although temperature is one of the most important environmental factors effecting metabolic rates of fish (Fry 1957). Likewise is the effect of body size on metabolic rates an important input parameter to growth, bioenergetics and population models (Kitchell *et al.* 1977).

Data about respiratory metabolism of juvenile or adult clupeids are rare, because these species are difficult to handle and known for their susceptibility to scale loss and handling stress in respirometers (Hettler 1976; Blaxter and Hunter 1982). Furthermore, some measurements with closely related species like *Sprattus fuegensis* (Chekunova and Naumov 1977) or *Clupea harengus* (Chekunova 1979) were conducted in closed respirometer systems, where oxygen partial pressure was measured only at start and end of an experiment. In such systems measurements are integrated over long times and it is impossible to isolate periods with no spontaneous activity representing standard metabolism. Standard metabolism is defined as the metabolism of a resting, unfed, but not starving fish, not influenced by spontaneous activity (Brett 1962) in contrast to routine metabolism, which includes increased oxygen consumption rates related to spontaneous activity (Fry 1947). In closed systems only an uncontrolled mixture of standard and routine metabolism can be measured, depending on the frequency of spontaneous activity of the experimental fish. Therefore we decided to perform our measurements in an intermittent flow respirometer, which was adjusted in dimensions to fish- and group size to allow short measuring intervals. Intermittent-flow systems are limited in terms of the measuring interval, which is shorter when the chamber volume is small in relation to fish size. The

shorter the measuring interval the more likely one can observe metabolic rates not influenced by spontaneous activities. Blaxter (1989) proposed using the lowest observed values from repeated observations as standard metabolic rate to exclude increased oxygen consumption due to spontaneous activity or stress. According to this proposal the 10%-lowest oxygen consumption rates have been used as the best approximation of standard metabolism in pelagic fishes (Herrmann and Enders 2000; Enders and Herrmann 2003; Ohlberger *et al.* 2007).

The aim of the present study was therefore 1) to determine metabolic rates of sprat in relation to water temperature and body mass and 2) to test if the 10%-lowest oxygen consumption rate is a reasonable approximation of standard metabolism for a permanently active fish like sprat. With these metabolic rate measurements the central component of a bioenergetic budget will be available allowing the future estimation of sprat daily rations and improved calculations of its predatory impact.

2.1 Material and Methods

2.1.1 Fish capture and maintenance

Adult sprat are commonly distributed in the deeper regions of the Baltic Sea, whereas the larvae and juveniles occur in the shallower coastal areas, where it is possible to catch them alive. We therefore caught Young of the Year (YoY) juvenile sprat in August 2005 and 2006 in Kiel Fjord (Wendtorf harbour) with a hand operated dip net (area: 4 m^2; mesh size 6 mm). Fish were transported to the aquarium facility at the University of Hamburg, Institute of Hydrobiology and Fisheries Science, where they were maintained in groups of 300-500 individuals in artificial seawater in circular tanks (diameter 1.5 m) under a 13L:11D light regime for at least two months before acclimation to experimental conditions. Photoperiod was identical in respirometer and rearing tanks. Routine phase measurements were conducted exclusively during the light phase. Tanks were connected to a recirculating system with a salinity of ~16, a temperature of 16°C and pH between 8.1 and 8.2. Fish were fed twice a day with dry food (LARVIVA, DANA FEED A/S) and once a day with live freshly hatched brine shrimp nauplii (*Artemia* sp., INVE Aquaculture). At least two weeks prior to the experiments groups of 25-30 animals were placed in circular tanks of a volume of 80-100 l for acclimation to experimental temperature and smaller tank size. Experiments were performed with two different size classes of sprat: small sprat with a mean standard length L_S of 68.8 ± 2.5 mm and a mean wet weight of 3.11 ± 0.19 g, and large sprat had a mean L_S of 92.6 ± 2.8 mm with a mean wet weight of 8.13 ± 0.94 g. Small fish were used two months after catch, whereas the larger fish lived more than one year in the aquarium of the institute. Because sprat is very sensitive to scale loss, it is not possible to measure fish size or weight before an experiment. We tried to visually select fish of similar body sizes for each experiment, but there remained a significant level of weight variation within each size class (small/ large). Given the high activity related variation in all experiments we decided to

contrast two size classes assuming that the size effect would be sufficiently large compared to variation within the size classes. To achieve this size difference of approximately 3 cm a group of the initially small sprat had to be maintained at high feeding levels for about a year in the aquarium. Since length growth is considerably slowed down in the second year it was not possible to conduct experiments with a third well separated size class.

2.1.2 Experimental Set-up

Measurements were conducted in a computer controlled intermittent-flow respirometer (Forstner 1983; Steffensen 1989; Herrmann and Enders 2000; Schleuter *et al.* 2007). The respirometer chamber had a volume of 99.9 l including all pipes and was submerged in a larger tank, which was connected to the seawater circulating system. The water in the outer tank was kept air-saturated by means of an air stone and water level was controlled by an overflow system. A cooling coil and two heating rods (SCHEGO 300) regulated the temperature in the outer tank. In addition a circulation pump (EHEIM) was placed in the outer tank for water mixing.

Each experiment lasted for at least 45 h with alternating refreshing- und measuring intervals. Measuring intervals lasted 23 min and were followed by refreshing-intervals of 5 min, when oxygen saturated water from the surrounding tank was pumped into the chamber to re-establish the oxygen-saturation to 95-100%. Within measuring periods the oxygen saturation and water temperature in the closed chamber were measured by a microprocessor oximeter (WTW, Oxi 539) and recorded once a second. After a short delay (3 min) allowing for homogenous mixing in the respirometer chamber the oxygen decline was interpreted as respiration rate. During refreshing periods the oxygen sensor was calibrated automatically for use in the following measuring interval. A flowmeter (B.I.O.-Tech, VISION2000) controlled the flow through rate (~ 1.74 l min^{-1}) in the respirometer chamber. All measurements were recorded on a PC in a separate room by the measuring software ARGUS (SORCUS Computer GmbH, Version 4.0), which also controlled all devices and displayed all variables during the experiments. Swimming behaviour of large sprat was observed via video camera and swimming speeds were detected by the use of an automated image analysing programme. These results and the complex image analysis methodology, however, are not part of the present publication. Swimming activities of small sprat were not filmed or analysed, but periodically observed.

2.1.3 Experimental Design

Fish were not fed 48 h before an experiment in order to avoid post feeding effects on metabolism. Oxygen consumption rates were measured for groups of schooling fish (n=6-25) instead of individuals, because individually separated clupeids are hyperactive, lose scales and die due to stress (Hettler 1976; Blaxter and Hunter 1982). At the end of an experiment fish were removed and rapidly killed by an overdose of anaesthetic (MS-222) and weighed

individually (0.001 g accuracy). For every single fish standard (L_S) and total length (L_T) were measured (0.1 mm below). After removing fish from the respirometer chamber the system was closed again without cleaning and oxygen uptake was measured for at least 24 h to determine bacterial respiration. Bacterial respiration was either constant or declined over approximately 4-10 hours, and was more or less stable for the remaining period. Relative surface area and material of the respirometer exert a considerable influence on bacterial respiration, whereas animal size affects bacterial growth only slightly (Da Villa 1983). Cases with declining bacterial respiration were caused by suspended matter, which had accumulated in the core zone of the tank with minimal current velocities during the experiments. During the process of fish removal this matter was stirred into the water column and resulted in initially increased bacterial respiration rates. Since this suspended matter originated from the water of the recirculation system and not from the unfed fish, the bacterial respiration was calculated in these cases from the period after the initially high values. Overall bacterial respiration accounted for 20-23% of total oxygen consumption depending on temperature. Values for bacterial respiration were subtracted from the total oxygen consumption rate of the proceeding experiments.

Altogether 21 experiments were performed at temperatures between 9 and 21°C using a total of 261 fish (see Table 2-1).

2.1.4 Data analysis

After fish were transferred into the respirometer chamber they firstly showed very high oxygen consumption due to handling stress, *i.e.* acclimation phase. This initial phase was not included in the data analysis. The separation between acclimation and routine phase followed the procedure of Herrmann and Enders (2000). For calculations of metabolic rates only data of the routine phase were used. Taking into account the chamber volume and the displaced volume of the fish, the actual oxygen uptake rate in one measuring interval in $mgO_2\ h^{-1}$ of the fish group was calculated by linear regression of oxygen concentration on time. Only oxygen consumption rates with a regression coefficient >0.9 times the average of all regression coefficients (~ 0.97) were selected to calculate metabolic rates (Herrmann and Enders 2000). The total oxygen uptake was then divided by numbers of fish and fish wet weight after values for bacterial respiration were subtracted.

2.1.5 Determination of metabolic level

Standard metabolism (R_S) was calculated as the median of the lower 10% of the respiration rates in the routine phase over one experiment (Herrmann and Enders 2000). For the calculation of routine metabolism (R_R) all oxygen consumption values of the routine phase have been used:

$$V_{O2}=\sum(V_i\Delta t_i)(\sum\Delta t_i)^{-1} \quad (1)$$

V_{O2}=average oxygen consumption in the routine phase ($mgO_2\ h^{-1}$); V_i=oxygen consumption of interval i in the routine phase ($mgO_2\ h^{-1}$); Δt_i =length of interval i in the routine phase (h).

2.1.6 Effect of body mass and temperature

The relationship between metabolic rate and water temperature was described by the following exponential relationship:

$$M'_{O2}=a\ e^{c\ T} \quad (2)$$

where M'_{O2} is the specific oxygen consumption of an unfed fish ($mg\ O_2\ g^{-1}\ h^{-1}$), and T the temperature (°C). The coefficients *a* and *c* were estimated using non-linear regression analysis based on a Levenberg-Marquardt-Algorithm (SPSS 17.0). Temperature effects were estimated for two sprat groups separately (see Table 2-1).
The combined effect of body mass and water temperature on metabolic rates was described using the following equation (Ware 1978):

$$M_{O2}=a\ W^{b}\ e^{c\ T} \quad (3)$$

M_{O2}=oxygen consumption ($mgO_2\ Ind^{-1}\ h^{-1}$), W=fish wet weight (g) and T = temperature (°C). The parameters *a*, *b* and *c* were estimated using non-linear regression.

Q_{10} values were determined using the following equation:

$$Q_{10}=RR_{(T+10)}\ /RR_T \quad (4)$$

$RR_{(T+10)}$ is the respiration rate at 10°C higher than respiration rate RR_T at temperature T. Q_{10} values can be calculated from *c*-values of equations (2) and (3) by $Q_{10}=e^{(c\ 10)}$.

Equations (2) and (3) were also fitted as global models with shared parameters *b* and/or *c* following the description in Motulsky and Christopolous (2004). With these fits it was tested if standard and routine rates (parameter *a*) differed significantly. The model was then formulated with two different parameters, a_{RR} and a_{RS} instead of *a*, and a conditional statement was included in the model formulation that links the respective subsets of the data (routine data and standard data) to the respective parameters. The two parameter values are not significantly different if the 95% confidence intervals (95%-CI) of one parameter (*e.g.* a_{RR}) include the other parameter estimate (a_{RS}) and vice versa. It was also possible to conduct an *F*-test for nested model comparison with the model outputs from two model fits, one with a shared parameter (nested model), and one with two separate parameters (full model; see Motulsky and Christopolous 2004 for details).

2.1.7 Comparison of data

Oxygen consumption rates of clupeoids from earlier investigations were compared to results from the present study by correcting routine respiration rates to a wet body weight of 10 g using the equation:

$$M_{O2\,(10)}=V_{orig}\,(BW/10)^{(1-b)} \quad (5)$$

where $M_{O2(10)}$ is the oxygen consumption rate ($mgO_2\ Ind^{-1}\ h^{-1}$) of a fish with 10 g wet weight, V_{orig} is the originaly measured oxygen consumption rate of a fish with wet weight BW while *b* is the mass exponent from equation (3). A value of *b*=0.86 according to Brett and Groves (1979) was chosen for all species where no specific information was available.

2.2 Results

2.2.1 Metabolic level

Routine metabolic rates were between 2% (Exp. 3 in Table 2-1) and 38% (Exp. 7 in Table 2-1) higher than standard metabolic rates of the respective experiments. The variability between experiments implies that some experiments were characterised by higher activity variations and that therefore temperature and weight effects should preferably be estimated from data on standard metabolism. The mean difference over all experiments was 12.8%. Global fitting of equation (2) to all routine and standard rate data of small sprat with shared parameter *c* and separate parameters a_{RR} and a_{RS} resulted in values of a_{RR}=0.074 for routine and a_{RS}=0.066 for standard metabolism, respectively (Table 2-2). For large sprat the equivalent fit resulted in parameter estimates of a_{RR}=0.094 for routine and a_{RS}=0.083 for standard metabolism. The differences between the metabolic levels amount to 12.1 and 13.2%, respectively. However, the 95% confidence intervals of a_{RS} include the estimate of a_{RR} and vice versa. Thus, both metabolic rates are not significantly different from each other, neither in small nor in large fish. *F*-statistics comparing the global fit with shared parameters a (nested model) with the model with separate estimates for a (full model) showed no significant differences between both models, neither in small (*F*=0.127; *p*=0.727) nor in large sprat (*F*=0.081; *p*=0.778).

If the same analysis with separate parameters a_{RR} and a_{RS} is performed based on equation (3) the data of small and large sprat can be analysed together, with additional shared parameters *b* (weight effect) and c (temperature effect, Table 2-3). This analysis resulted in parameter estimates of a_{RS}=0.067 for standard and a_{RR} =0.076 for routine metabolism, respectively. The differences between the metabolic level amount to 11.8%. Again, the 95% confidence intervals indicate that standard and routine metabolic rates are not significantly different from each other and *F*-statistics showed no differences between

the nested model with shared parameter estimates and the full model with separate estimates for parameter *b* and *c* (F=0.093; p=0.762).

Table 2-1 Routine (R_R) and standard (R_S) respiration rates for sprat at different temperatures (T, °C) with the corresponding individual mean wet weights and number of fish used in each experiment.

Exp No	*T* [°C]	*RP* [h]	Number of fish [n]	Mean wet weight ± SE [g]	Mean R_R ($mgO_2\ g^{-1}\ h^{-1}$)	Mean R_S ($mgO_2\ g^{-1}\ h^{-1}$)	Difference R_R-R_S [%]
1	9.6	63	19	3.16 ± 0.72	0.169 ± 0.032	0.134 ± 0.005	26
2	9.7	20	15	2.98 ± 0.72	0.162 ± 0.032	0.137 ± 0.002	18
3	12.4	30	25	3.26 ± 0.51	0.162 ± 0.017	0.159 ± 0.001	2
4	12.7	32	18	2.70 ± 0.72	0.215 ± 0.030	0.193 ± 0.004	11
5	15.1	22	20	3.14 ± 0.98	0.213 ± 0.029	0.197 ± 0.002	8
6	15.3	35	12	3.11 ± 0.58	0.241 ± 0.100	0.229 ± 0.003	5
7	15.3	10	19	2.82 ± 0.74	0.272 ± 0.042	0.197 ± 0.008	38
8	18.1	17	17	3.26 ± 0.74	0.298 ± 0.010	0.287 ± 0.002	4
9	20.8	14	18	2.86 ± 0.60	0.367 ± 0.014	0.328 ± 0.001	12
10	10.3	14	8	8.79 ± 1.99	0.188 ± 0.020	0.184 ± 0.005	2
11	13.2	24	9	8.38 ± 3.56	0.203 ± 0.016	0.197 ± 0.003	3
12	13.3	64	10	8.11 ± 2.51	0.267 ± 0.023	0.248 ± 0.005	8
13	16.2	25	7	6.76 ± 1.45	0.371 ± 0.080	0.285 ± 0.003	30
14	16.2	23	6	7.75 ± 2.63	0.350 ± 0.060	0.285 ± 0.003	23
15	16.2	20	7	6.76 ± 1.45	0.322 ± 0.059	0.280 ± 0.006	15
16	16.3	46	8	8.30 ± 2.63	0.370 ± 0.032	0.308 ± 0.004	20
17	16.3	18	11	8.07 ± 1.68	0.323 ± 0.033	0.304 ± 0.003	6
18	18.8	28	7	9.71 ± 2.28	0.311 ± 0.025	0.273 ± 0.006	14
19	19.1	43	10	8.45 ± 2.70	0.504 ± 0.044	0.456 ± 0.004	11
20	19.1	39	7	9.08 ± 2.23	0.385 ± 0.025	0.335 ± 0.006	15
21	19.1	18	8	6.65 ±1.57	0.376 ± 0.022	0.358 ± 0.003	5

Exp No= consecutive number of experiments, *RP* = time of the routine phase in hours of each experiment, only values from these phases were used for calculations of metabolic rates. Difference R_R- R_S = Difference between R_R and R_S as percentage of the standard metabolic rate. Experiments 1-9 were conducted with small sprat, experiments 10-21 were undertaken with large sprat

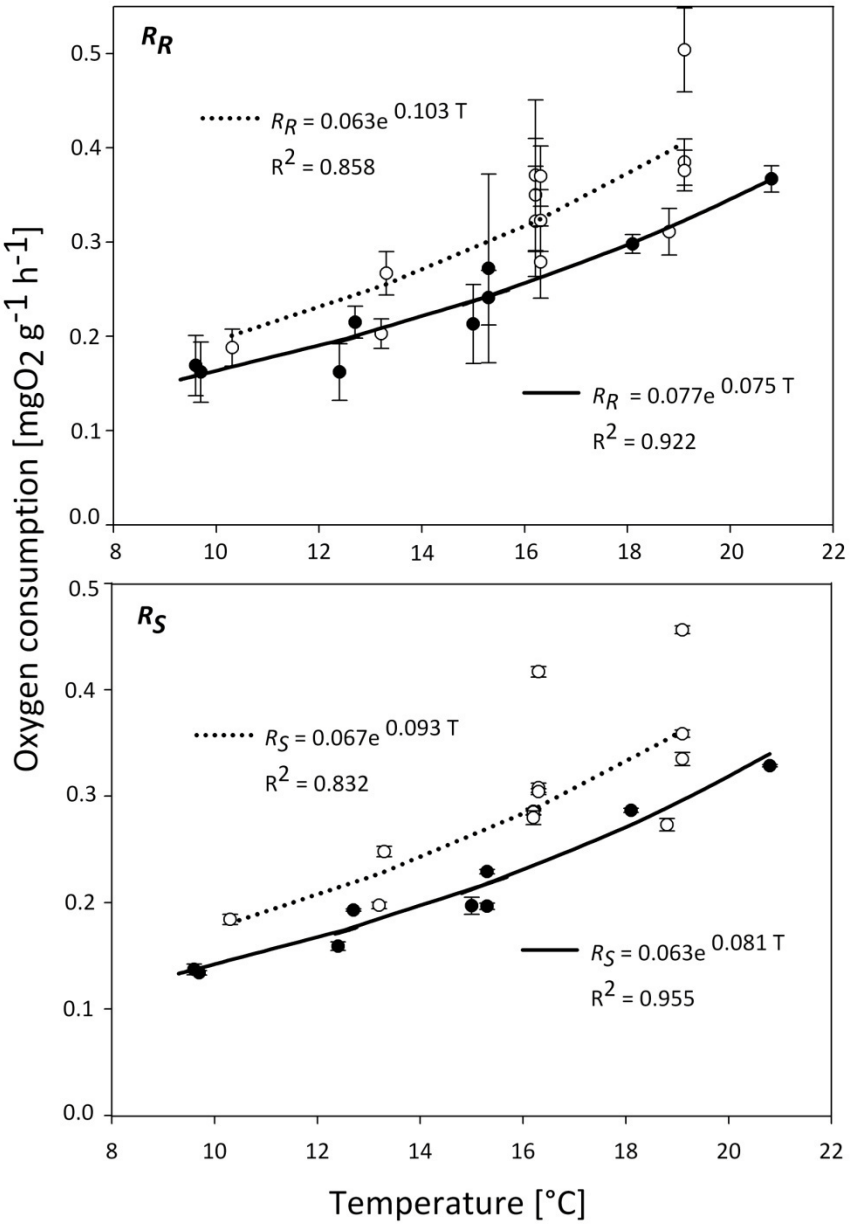

Figure 2-1 Routine (R_R) and standard (R_S) metabolic rates (mg O_2 g^{-1} h^{-1}) of small (●) and large (○) sprat in relation to water temperature (°C). Regression equation and statistics are provided in Table 2-2

2.2.2 Body mass effect

Metabolic scaling exponents were estimated separately for standard and routine metabolic rates based on equation (3) as *b*=1.077 (routine) and *b*=1.073 (standard, Table 2-3). The confidence intervals in both cases included the case of isometric scaling (b=1.0). Not surprisingly, a shared weight scaling exponent *b* from a fit of equation (3) with two parameters a_{RR} and a_{RS} and shared parameters *c* and *b* revealed a scaling exponent of *b*=1.074 with confidence intervals still including *b*=1.0. The respiration rates in experiment 19 at 19.10°C for large sprat were much higher than the other measurements at the same temperature (Table 2-1). The removal of this data point, however, did not result in

significantly different parameter estimates for *a*, *b* and *c* in a fit of equation (3) to the whole data set (4% difference for R_S and 2.5% for R_R).

Table 2-2 Parameter estimates with the corresponding 95%-Confidence intervals (CI) of the non-linear regression analysis $M'_{O2} = a\, e^{c\,T}$, relating routine (R_R) and standard (R_S) metabolic rates ($mgO_2\ g^{-1}\ h^{-1}$) of small (~3.1 g) and large (~8.5 g) sprat to temperature (T, °C). R^2 is the coefficient of determination. Global fitting ($R_{R,S\ GLOBAL}$) was undertaken to compare both activity level with shared *c*

Fish-group	Exp No	Metabolic level	*a* ± SE	95%-CI		*c* ± SE	95%-CI		R^2	Q_{10}
small	1-9	R_R	0.077 ± 0.010	0.052	0.101	0.075 ± 0.008	0.056	0.094	0.922	2.12
		R_S	0.063 ± 0.007	0.046	0.079	0.081 ± 0.007	0.065	0.096	0.955	2.25
		$R_{R\ GLOBAL}$	0.074 ± 0.006	0.060	0.087					
		$R_{S\ GLOBAL}$	0.066 ± 0.006	0.054	0.078	0.078 ± 0.005	0.067	0.088	0.941	2.18
large	10-21	R_R	0.088 ± 0.030	0.021	0.156	0.080 ± 0.020	0.036	0.124	0.672	2.23
		R_S	0.080 ± 0.025	0.023	0.137	0.079 ± 0.018	0.038	0.120	0.684	2.20
		$R_{R\ GLOBAL}$	0.094 ± 0.022	0.048	0.140					
		$R_{S\ GLOBAL}$	0.083 ± 0.019	0.043	0.122	0.077 ± 0.013	0.049	0.105	0.698	2.16

Exp No = number of experiments used for analysis

2.2.3 Temperature effects

For small sprat (experiments 1-9, Table 2-1) routine metabolism R_R increased from 0.162 mg O_2 g^{-1} h^{-1} at 9°C to 0.367 mg O_2 g^{-1} h^{-1} at 21°C. Standard metabolism, R_S, increased in small sprat from 0.137 to 0.328 mg O_2 g^{-1} h^{-1} at 9°C and 21°C, respectively (Figure 2-1). Routine metabolism in large sprat (experiments 10-21, Table 2-1) increased from 0.188 to 0.504 mg O_2 g^{-1} h^{-1} at 10°C and 19°C, respectively, and R_S increased from 0.184 to 0.456 mg O_2 g^{-1} h^{-1} at 10°C to 19°C, respectively (Figure 2-1, Table 2-1).

Q_{10} values (Table 2-2) for routine metabolism ranged from 2.12 (*c*=0.075) for small sprat to Q_{10}=2.23 (*c*=0.080) for large sprat. For standard metabolism Q_{10} values ranged from 2.25 (*c*=0.081) for small sprat to 2.20 (*c*=0.079) in large sprat. Since the confidence intervals of the estimated temperature coefficients of small sprat include the *c*-estimate for large sprat and vice versa, we have combined the data and estimated one shared temperature coefficient. The overall analysis of all data using equation (3) revealed a temperature coefficient of c_{RR}=0.080 for routine and c_{RS}=0.078 for standard metabolism, respectively.

2.3 Discussion

2.3.1 Determination of metabolic level

The metabolic level estimated as the 10% percentile of the measurements during the routine phase is only 13.4 % lower than the routine (=median) metabolic rate for the

combined analysis (Table 2-3). Confidence intervals of the parameters in global models (Table 2-2 and 2-3) and *F*-tests comparing nested models indicated that this difference was not significant. However, due to the low number of independent observations that can be obtained with group experiments and the number of parameters needed in the global models, the lack of significance is also not surprising. The small difference between standard and routine rates reflects mainly the limited range of variation in sprat activity levels. Sprat showed permanent swimming activities during experiments, what could be referred to as routine swimming, because fish were not feeding and they were not forced to swim against a strong current. Large sprat showed higher activities at the start of an experiment, but mean swimming speeds changed very little during the routine phase and ranged from 3.0 to 8.0 cm s^{-1}. For small sprat no automated video analysis was undertaken, but fish were observed periodically and the same sustained swimming behaviour was noted. Only at the start of an experiment some fish showed very high swimming speeds and increased turning rates.

Table 2-3 Parameter estimates with the corresponding 95%-Confidence intervals (CI) and the coefficient of determination (R^2) of the non-linear regression function, $M_{O2} = a\ W^{b}\ e^{c\ T}$, relating routine ($R_R$) and standard metabolic (R_S) rates ($mgO_2\ Ind^{-1}\ h^{-1}$) to body wet weight (W ,g) and water temperature (T, °C) for *Sprattus sprattus* from all experiments. Global fitting ($R_{R,S\ GLOBAL}$) was undertaken to compare both activity level with shared parameters *c* and *b*

Metabolic level	*a* ± SE	95%-CI		*b*± SE	95%-CI		*c* ± SE	95%-CI		R^2	Q_{10}
R_R	0.074 ± 0.025	0.021	0.128	1.077 ±0.131	0.802	1.353	0.080±0.015	0.048	0.111	0.917	2.23
R_S	0.069 ± 0.022	0.022	0.116	1.073 ±0.124	0.812	1.334	0.078±0.014	0.048	0.108	0.923	2.18
$R_{R\ GLOBAL}$	0.076 ± 0.018	0.041	0.111								
				1.074 ±0.088	0.896	1.253	0.079±0.010	0.058	0.099	0.920	2.20
$R_{S\ GLOBAL}$	0.067 ± 0.016	0.036	0.099								

In the present investigation it was not possible to reduce measuring intervals below 23 min, because the chamber size needed to be large enough for sprat groups to move naturally. Thus, we were able to undertake repeated measurements of calmed sprat, but due to these long intervals and the use of groups it was not possible to separate intervals with nearly no spontaneous activities and very low metabolic rates. In some of the experiments occasional periods of increased activity occurred and the contribution of these was effectively eliminated by use of the percentiles, as is visible in large differences between routine and standard rates of up to 38% (Table 2-1). Since in other experiments the difference was as low as 2% the standard rate data should generally be a more consistent data base for the estimation of temperature and weight effects. However, small differences between standard and routine metabolism do not necessarily indicate a lower activity level in these experiments, but only that the activity level was not variable between the 23 min measuring intervals.

In horse mackerel standard metabolic rates determined with percentile analysis were on average 24.4% lower than routine rates and thus more clearly separated (Herrmann and Enders 2000). However, horse mackerel were held as individuals during experimentation and thus, very low metabolic rates were detectable. Due to the smaller tank size in relation to fish mass, the measuring intervals in Herrmann and Enders (2000) were only 5 min long, and repeatedly periods with only very limited fin movements were recorded. In the present study standard metabolism after percentile analysis measured for fish groups may not correspond to the definition of standard metabolism by Brett (1962) or Fry (1971) as metabolism of a resting, unfed fish, not influenced by spontaneous activity. Jobling (1994) suggested to term low metabolic rates influenced by slight spontaneous swimming activities as *fasting metabolic rate*. Based on this definition the presently calculated standard metabolic rate (R_S) of sprat could be referred to as *fasting metabolic rate*.

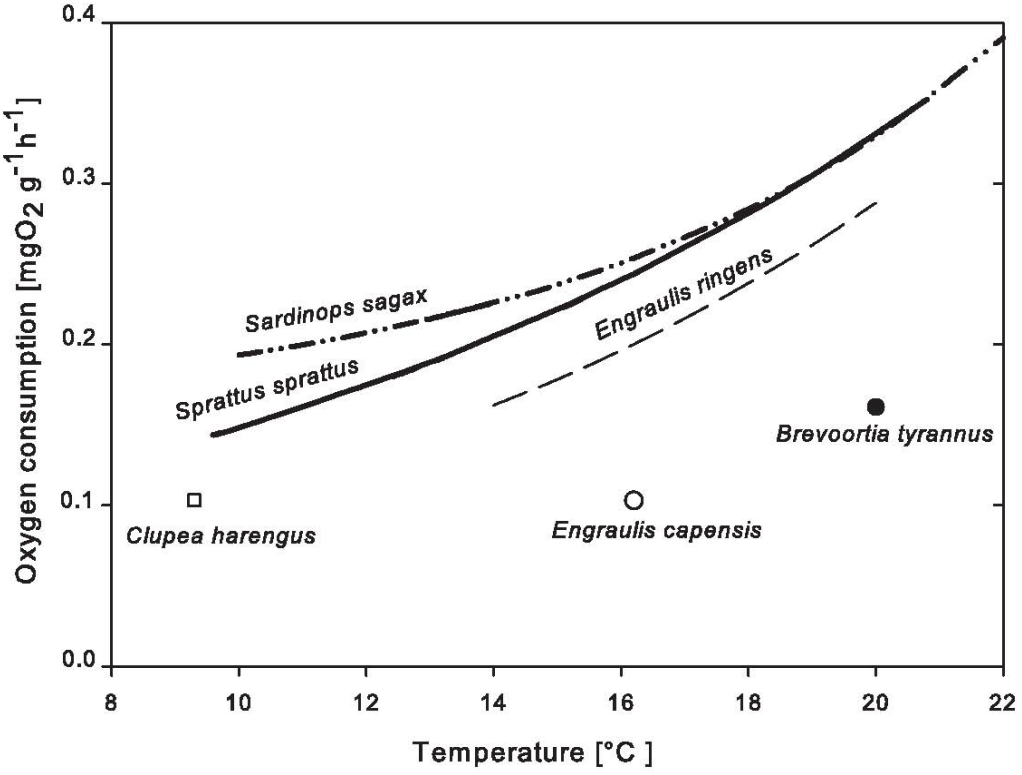

Figure 2-2 Relationship between routine respiration rate ($mgO_2\ g^{-1}\ h^{-1}$) and temperature (°C) of sprat from the current study compared to published routine rates for other fishes within Clupeiformes. Sprat respiration rate was determined by the use of the regression function from the combined analysis (Table 2-3). All values have been corrected to a wet body weight of 10 g using Eq. (5). Regressions and values are for directly measured metabolic rates except for *E. capensis*, which has been calculated by extrapolating to a swimming speed of 1 body length s^{-1}. See text for further details

Standard metabolic rates of clupeids have often been estimated through extrapolation to zero swimming speed (Durbin *et al.* 1981; Villavicencio 1981; van der Lingen 1995) or by the use of neuromuscular blocking agents, which prevent any muscle activity (Leonard *et al.* 1999). For *Sardinops sagax* (van der Lingen 1995), for instance, extrapolating routine

metabolic rate to zero swimming speed yielded in an extremely low oxygen consumption rate of 0.009 mg O_2 g^{-1} h^{-1}, which was also noticed as "basal" metabolic rate in the respective study. Estimated standard metabolism of adult B. tyrannus at 20°C with the extrapolation method revealed rates of 0.036 - 0.089 mg O_2 g^{-1} h^{-1}, which are substantially higher than the rate of S. sagax (van der Lingen 1995), but also much lower than the respective lowest observed metabolic rates (Durbin *et al.* 1981; Macy *et al.* 1999).

Concluding, oxygen consumption rates of anaesthetized animals as well as those extrapolated to zero swimming speed seem to characterise rather the basal metabolism described for homeothermic animals (Blaxter 1989) than a standard metabolic rate.

2.3.2 Temperature effects

An accurate parameterisation of the temperature effect on metabolic rates is a precondition for the application of bioenergetic models of fish in habitats with strong seasonal temperature fluctuations. The results from the present investigation demonstrate that temperature has a significant effect on the metabolic rate of sprat, resulting in a Q_{10} of 2.2 for both standard and routine metabolism (Table 2-3). This is in agreement with findings of previous studies of related species: Leonard *et al.* (1999) obtained a Q_{10} of 2.2 for adult (~ 40 cm) American shad (*Alosa sapidissima* Wilson), an anadromous clupeid, between 15.6 and 21.3°C. A similar Q_{10} value of 2.1 can be determined for juvenile (75 to 81g) Atlantic menhaden using the data Hettler (1976) measured over a temperature range from 10 to 25°C. Higher Q_{10} values have been reported for juvenile (8.5 cm) sardines (*Sardinops sagax* L.) between 15 and 20°C (Q_{10}=3.2; Villavicencio *et al.* 1981) and juvenile (12 cm) anchoveta (*Engraulis ringens* L.) between 14 and 20°C (Q_{10}=2.6; Villavicencio 1981). Van der Lingen (1995) found a somewhat lower Q_{10} value of 1.82 over a broader range of temperatures (9.7 to 22.7°C) for adult (25.6 cm) *S. sagax*, possibly indicating an adaptation to the upwelling area of the Benguela current, where low temperatures correlate with a high food availability. A low Q_{10} value ensures in this case that metabolism is not strongly down regulated in periods of high food supply. By contrast, in boreal waters like the Baltic Sea low temperatures occur during winter, when zooplankton density is low. However, for a conclusive interpretation of metabolic Q_{10} values additional information would be needed on the respective Q_{10} values of food intake rates.

Routine metabolic rate of 10 g sprat from the present investigation was compared to routine rates determined for other clupeoids (see Figure 2-3). The routine respiration rate of sprat at 18.1 °C equals the routine metabolism determined for *S. sagax* at 18°C (van der Lingen 1995, Figure 2-3). Deviations increase below a temperature of 18°C due to the lower Q_{10} value for *S. sagax* of 1.82. Considerably lower routine metabolic rates have been found for juvenile (5.9 g) European anchovy *Engraulis encrasicolus* L. (James and Probyn 1989) and juvenile (12.6 g) anchoveta *E. ringens* (Villavicencio 1981) standardized for 10 g fish. Also for the Atlantic menhaden *B. tyrannus* (Durbin *et al.* 1981) routine metabolism was much lower than in sprat from the current study (Figure 2-3). This might be explained by the fact, that *B.*

tyrannus is an obligate filter-feeding clupeid, whose metabolism is influenced by activities predominately during feeding periods, but shows very low swimming activities during non-feeding phases (Durbin *et al.* 1981). The routine respiration rate of *C. harengus* (Johnstone *et al.* 1993) at 9.3°C of 0.103 $mgO_2\ g^{-1}\ h^{-1}$ was also much lower than the metabolic rate of sprat. This could be explained by the huge size differences between sprat in our study and herring in the study of Johnstone *et al.* (1993) and differences in experimental design allowing for different swimming activities. Altogether the presently determined routine metabolic rate of sprat is higher than the majority of routine rates of closely related fish, but similar to that of *S. sagax*.

2.3.3 Body mass effect

The overall analysis of both body mass and temperature effects resulted in a weight exponent of *b*=1.077 (Table 2-3), which is substantially higher than the majority of weight scaling exponents for fish reported in the early literature. Winberg (1960) estimated a mean weight scaling exponent of *b*=0.81, and also later studies revealed a similar mean value of *b*=0.86 (Brett and Groves 1979; Jobling 1994; Steffensen 1994). Clarke and Johnston (1999) showed that scaling exponents vary between species, taxonomic families and different methodologies. However, weight exponents also tend to vary within a species depending on activity level during measurements, resulting in an increase of *b* with increasing activity (Brett 1965; Wieser 1985; Glazier 2009). Brett (1965) measured metabolic rates of sockeye salmon, typically showing permanent activity, at different swimming speeds and determined a scaling exponent of *b*=0.78 for low swimming speed and *b*=0.98 for maximum activity. Likewise measurements with chased northern pike *Esox lucius* L., a typical sit and wait predator, resulted in an increase of the scaling exponent from *b*=0.8 under routine conditions to *b*=0.99 under maximum activity (Armstrong *et al.* 1992). Generally interspecific differences in metabolic scaling can also be related to the species specific lifestyle: low weight scaling exponents of routine metabolism have been found for less active, demersal species like the plaice (*Pleuronectes platessa* L.) with *b*=0.626 (Jobling 1982) or the turbot *Psetta maxima* L. with *b*=0.699 (Waller 1986), whereas higher weight exponents have been found for more active, pelagic fish species like the skipjack tuna, *Katsuwonus pelamis* L., with values ranging from *b*=0.9 to 1.2 (Gooding *et al.* 1981). A similar relation between life style and metabolic scaling exponents was demonstrated for anaerobic metabolism of burst swimming in a comparison of benthic and pelagic fish species (Childress and Somero 1990; Somero and Childress 1990). An apparent exception from this pattern are the low exponents from *b*=0.496 to 0.573 determined for different tuna species in the studies of Brill (1979, 1987), but these were obtained from fish paralysed with a neuromuscular blocking agent. Likewise Leonard *et al.* (1999) described a low *b*-value of 0.695 for the anadromous clupeid *A. sapidissima* after the application of a neuromuscular blocking agent.

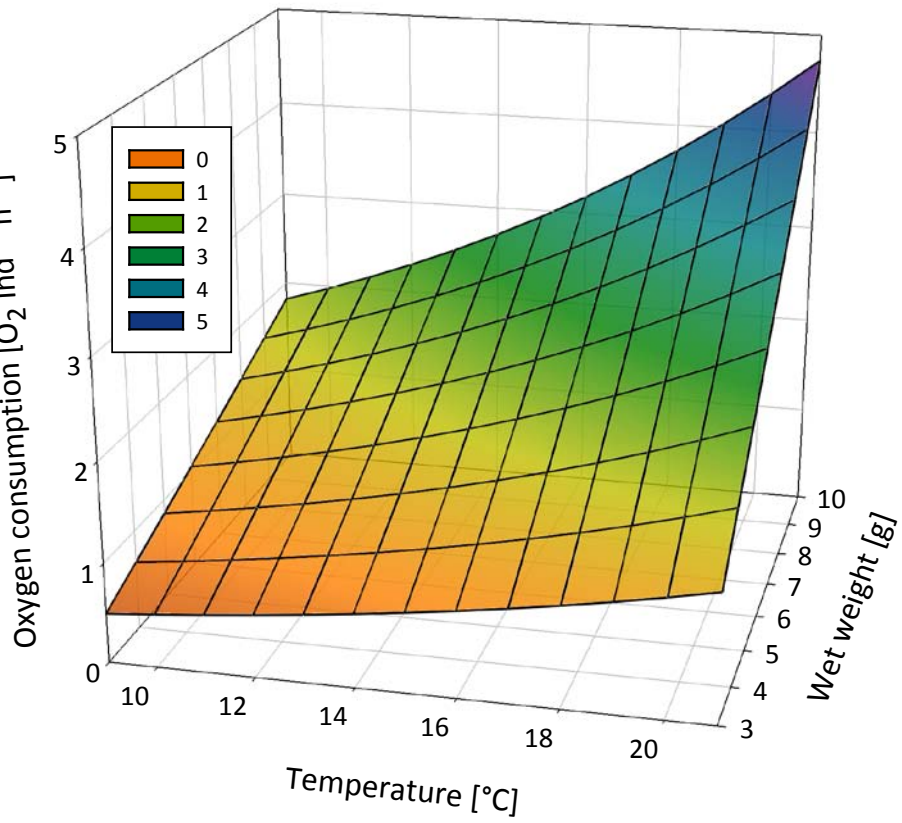

Figure 2-3 Routine metabolic rate of sprat (mgO_2 Ind^{-1} h^{-1}) in relation to wet weight (g) and water temperature (°C). Data are calculated by the use of the regression function resulting from the combined analysis (Eq. 3). For details see Table 2-3

Clupeids are active schooling fishes, which swim more or less continuously throughout the day (Blaxter and Hunter 1982), and consequently high weight scaling exponents have been described for several clupeids: Chekunova and Naumov (1977) obtained an exponent of *b*=1.01 for the Falkland sprat (*Sprattus fuegensis* Jenyns) with a weight ranging from 6.9 to 70 g. For Baltic herring (*Clupea harengus membras* L.) weighing 8.9 to 104.5 g Chekunova (1979) found a weight exponent of *b*=0.978. Stolbov (1992) determined a weight exponent of *b*=0.873 (± 0.074) for the standard metabolism of Black Sea sprat (*Sprattus sprattus phalericus* L.) weighing 2-9 g.

In addition to activity also SDA-effects tend to cause *b* approach 1 (Glazier 2009), but since fish in the present study were entirely fasted, this does not apply here. Furthermore some experimental studies revealed a significant effect of temperature on metabolic scaling (Glazier 2005), resulting in lower mass scaling exponents at higher temperatures (Ohlberger *et al.* 2007; 2008). Due to our experimental design, however, we cannot conclude if this aspect is of relevance in sprat. Allometric weight exponents of fish may also vary depending on the weight range and ontogenetic stage covered in experiments (Post and Lee 1996). Exponents referring to early life stages only, tend to be higher than those based on juvenile

and adult stages (Riisgard 1998; Glazier 2005). However, we investigated a total length range of L_T=8.1 – 12.5 cm, clearly excluding larval stages (L_S < 4.4 cm, ~ L_T < 5.2 cm; Peck *et al.* 2005) but extending near L-infinity which varies - depending on habitat - between 10 and 13 cm L_T (Fishbase 2009). From this it can be concluded that the permanently elevated activity level is the most likely reason for the high metabolic scaling exponent in sprat. The isometric metabolic scaling is in agreement with findings of studies on related fish species and should be taken into account in bioenergetic budget models.

For such bioenergetic budgets we suggest to use our standard metabolic rates, which include the basic activity level typical of pelagic schooling fish. The routine rates in many experiments were identical or very similar to the standard rates, but in some cases deviations occurred of up to 38%. Therefore the routine rates of our study are less consistent. For an application to field conditions a correction of the standard rates is needed to account for the additional activity related to feeding behaviour. Therefore an activity multiplier should be chosen in the lower range of typically used values between 1 and 2 (Winberg 1960; Kitchell *et al.* 1977; Hewett and Johnson 1992). Metabolic parameters of the present investigation differ considerably from previous estimates used in the bioenergetics budget for Baltic sprat (Arrhenius 1993), which was largely relying on parameter values from related species (Rudstam 1988). The improved estimates from the present study allow a more accurate assessment of the predatory impact of the large sprat stocks in the Baltic and North Sea ecosystems.

2.4 Acknowledgments

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3 Manuscript 2: Energy costs of spontaneous swimming in sprat, *Sprattus sprattus* L., at different water temperatures

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Abstract

To assess the energy costs of spontaneous swimming in the small clupeid sprat (*Sprattus sprattus* L.), we measured oxygen consumption rates of fish groups at a range of temperatures (10-19°C) in an intermittent-flow respirometer. Swimming speeds and turning rates were detected from digital images by an automated fish-tracking program. We evaluated to what extent the data from the initial phase of such experiments could be used to quantify the energy costs of spontaneous swimming patterns in sprat. We were able to fit a model relating oxygen consumption to mean swimming speeds and sharp turns (>90°), which are common behavioural elements of clupeid fish. Swimming speeds and turning rates did not change among the tested temperatures, but Q_{10} values increased with increasing activity. Metabolic costs for spontaneous swimming and turning rates can now be included as variables in bioenergetics models for sprat.

Keywords: respiration, Sprat, *Sprattus sprattus*, spontaneous activity, swimming performance, metabolism

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3.1 Introduction

European sprat, *Sprattus sprattus* L., is a small schooling clupeid fish and one of the most important pelagic resources in the Baltic Sea (Cardinale and Arrhenius 2000), both as target for fisheries (ICES 2010) and as dominating prey for valuable piscivores like cod *Gadus morhua* L. and Atlantic salmon *Salmo salar* L. (Sparholt 1994). Furthermore, sprat has a distinct influence on the zooplankton community in the Baltic Sea by size and species selective feeding (Rudstam 1994; Möllmann *et al.* 2004) and partly controls the recruitment success of cod by egg predation (Köster and Möllmann 2000). Despite its high ecological importance and the huge stock sizes in the Baltic Sea (ICES 2010), estimates on sprat specific food consumption and growth rates are not available for adults or late juveniles to assess the potential for top-down and bottom-up control within the pelagic food web.

The estimation of food consumption in fish is difficult because direct observations in the natural habitat are almost impossible. Therefore bioenergetics models were developed in order to indirectly estimate fish growth and consumption rates (*e.g.* Kitchell *et al.* 1977; Rudstam 1988; Hewett and Johnson 1992), but models for sprat were so far based on parameter estimates derived largely from related or even unrelated species (Arrhenius and Hansson 1993; Arrhenius 1998). Likewise were previous models for the closely related herring, *Clupea harengus* L., mainly based upon parameter estimates borrowed from other species (Ney 1993; Bernreuther 2007). However, measurements of species specific metabolic costs make modelled field estimates of food consumption or growth rates more robust. It is therefore of great importance to directly investigate metabolic rates of sprat in laboratory experiments. The most important factors influencing metabolic rates of fish are temperature (Jobling 1994), body mass (Ware 1978) and activity level (Brett 1964; 1965; Koch and Wieser 1983; Boisclair and Legett 1989). Consequently, metabolic rates of fish are separated into standard, routine and active metabolic rates (Fry 1971), depending on the swimming activity of fish during measurements. Thereby standard metabolism (R_S) describes the metabolic rate where the fish is at complete rest (Brett 1962), whereas routine metabolism (R_R) includes measurements at spontaneous swimming activities (Fry 1957) and active metabolic rates (R_A) are measured at maximum sustainable swimming speeds (Brett and Groves 1979). Thus, active metabolic rates represent the energy costs of fish swimming with more or less constant high speeds. These rates were mainly measured in swimming respirometers (Brett 1964; Beamish 1978; Gehrke *et al.* 1990), where fish are not allowed to show their complete swimming behaviour as they are forced to swim against a constant current. In some cases metabolism can be higher than the active metabolic rate, if for example fish are forced to swim at their maximum possible speeds. This metabolism is then termed maximum metabolic rate (MMR), but can be maintained only for short time periods. The intermittent swimming of spontaneously active fish involving angular accelerations like frequent turns is hydrodynamically less efficient than the uniform caudal locomotion of continuous swimming and represents therefore potentially higher metabolic costs than for continuous straight-line swimming (Smit 1965; Krohn and Boisclair 1994; Enders and

Herrmann 2003) or burst-and-coast swimming. The latter also involves acceleration and deceleration, but can be more efficient than steady continuous swimming (Videler and Weihs 1982). Movement patterns like turning manoeuvres and high accelerations are major components of spontaneous swimming activities (Tudorache *et al.* 2009; Steinhausen *et al.* 2010) and are supposed to represent common behaviours of pelagic fish during feeding (Brachvogel *et al.* 2012) searching for food or escaping from predators (Videler 1993).

Previous measurements of standard or routine metabolic rates of pelagic fishes were often conducted in intermittent-flow respirometers (Steffensen 1989) with preferably short measuring intervals. All measurements from initial experimental phases (acclimation phases) were thereby rejected for further analysis as these were largely influenced by variable swimming activities (Herrmann and Enders 2000; Olberger *et al.* 2007; Meskendahl *et al.* 2010). However, reliable estimates of spontaneous swimming costs actually require variable swimming activities of the fish with frequent changes in speed and high rates of manoeuvring (*e.g.* Tudorache *et al.* 2009; Steinhausen *et al.* 2010). Thus, the purpose of the present study was to utilize the measurements from acclimation periods, without the initial period of recovery from handling stress. During acclimation phases spontaneous swimming patterns were more variable than during routine phases, which are typically used for the estimation of standard and routine metabolic rates. In order to utilize these measurements adequately, one needs to monitor the observed swimming patterns simultaneously. Previous investigations revealed that oxygen consumption rates of fish correlate with various movement patterns like turning rates (Krohn and Boisclair 1994), tail beat frequency (Leonard *et al.* 1999; Steinhausen *et al.* 2005; Blank *et al.* 2007), pectoral fin beat frequency (Tudorache *et al.* 2009), tail beat angle (Enders and Herrmann 2003) or tail beat pressure (Steinhausen *et al.* 2007). Costs for these movement patterns were, however, not quantified in detail in the cited studies as no standard program for the automated tracking of schooling fish is available Previous programs for the automated detection of fish swimming behaviour in small tanks like for instance the system by Kaufmann (1983) for the detection of swimming activities of single fish or the more recent program of Pinkiewicz (2008) allowing the tracking of a fish school were, however, not directly applicable to our experimental design and fish species. We therefore developed a new system based on available algorithms and concepts for multiple object tracking, but adjusted for the present tank design, fish size, group size, visibility of fish in the respirometer chamber and the actual frame rate. This allows for the first time the detection of different movement patterns of sprat during a respirometry experiment.

The main objectives of the present investigation were therefore (1) to quantify the spontaneous swimming costs of sprat using oxygen consumption rates and simultaneous measurements of swimming speeds and direction changes and (2) to present a new approach for the application of measurements obtained shortly after the introduction of fish in a respirometer.

3.2 Materials and Methods

3.2.1 Fish capture and maintenance

Young of the Year (YoY) juvenile sprat were caught in the Kiel Bay (Baltic Sea, 54° 29'N; 10° 15'E) by a hand operated dip net with a mesh size of 6 mm. Fish were then transferred within a 700 l circular tank with aerated seawater to the aquarium facility at the University of Hamburg, Institute of Hydrobiology and Fisheries Science. Sprat were held in large groups in circular tanks (diameter 1.5 m) connected to the recirculating system of the institute at a salinity of 16 and a temperature of 16°C more than twelve months prior to the start of experiments. Fish were fed twice a day with pellet food (LARVIVA, DANA FEED A/S) and once a day with live freshly hatched brine shrimp nauplii (*Artemia* sp.; INVE Aquaculture).

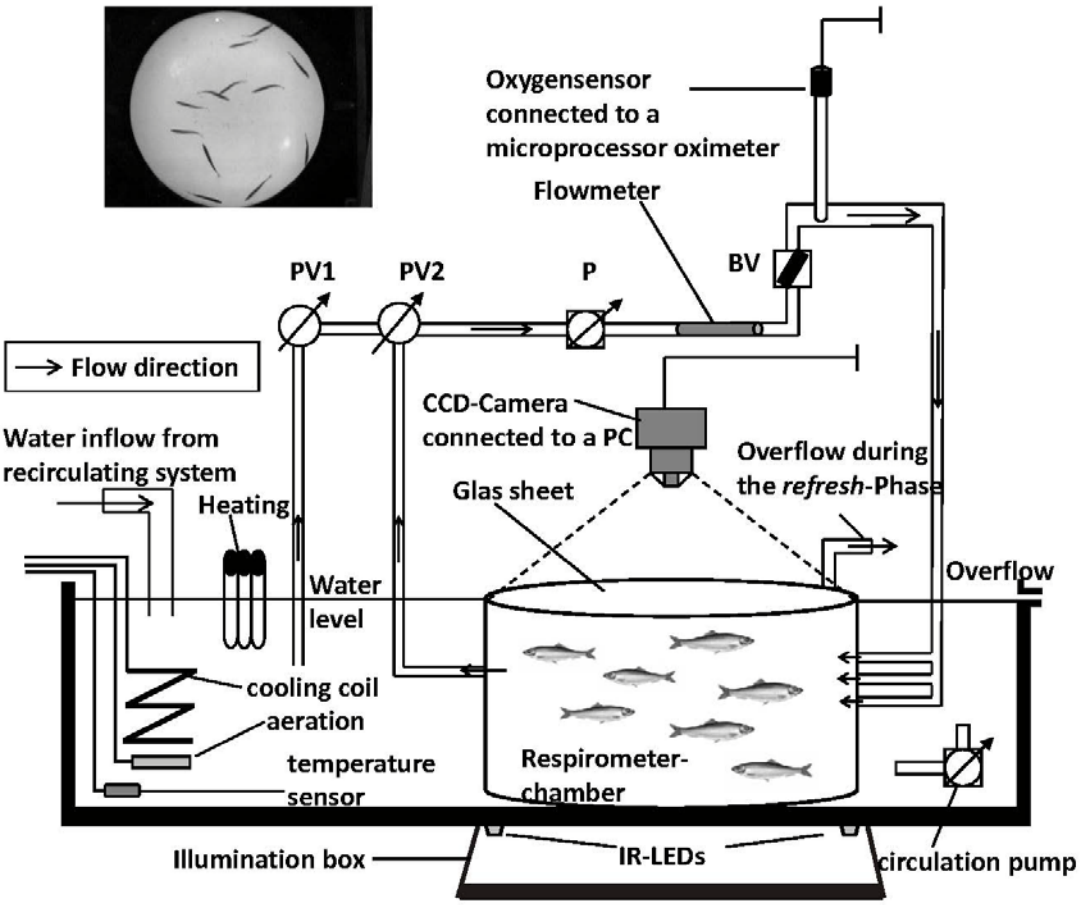

Figure 3-1 Schematic of the intermittent-flow respirometer for measuring metabolic rates of sprat with simultaneous image recordings. Images were captured with a frame rate of 4 Hz by use of a CCD Camera connected to a PC. Illumination for the Camera was given by infrared-LEDs under the respirometer chamber. This allowed a bright and constant background for which fish appeared as dark objects. PV1 and PV2 are computer controlled pneumatic valves. During the measuring phases (23 min) PV1 was closed and opened during *refresh*-Phases (5 min), so that oxygen-saturated water from the surrounding tank was pumped into the respirometer chamber. P = pump, BV = ball valve

3.2.2 Respirometry and experimental design

Fish were acclimatised to experimental temperature (10, 13, 16 or 19°C) and smaller tank size (80-100 l) for two weeks and were not fed 48 h prior to experimentation in order to avoid post feeding effects on metabolism. The influence of temperature and activity on metabolic rates of sprat was quantified by measuring oxygen consumption of a group of 8-13 fish in an intermittent-flow-respirometer (Steffensen 1989; Schleuter *et al.* 2007; Meskendahl *et al.* 2010) with a chamber volume including all pipes of 99.9 l The circular respirometer chamber itself had a radius of 27 cm and a height of 39 cm and was submerged in a larger tank, connected to the seawater circulating system (Figure 3-1). The chamber dimensions allowed measuring a significant decrease in oxygen consumption of a small fish group over a preferably short measuring time. A flowmeter (B.I.O.-Tech, VISION2000) was inserted between the pump and the oxygen sensor in order to adjust the water flow rates through the chamber, which indirectly controlled the water velocity. All measurements, including the flowmeter count (l min^{-1}), were recorded on a PC in a separate room by the measuring software ARGUS (SORCUS Computer GmbH, Version 4.0), which also controlled all devices and monitored all variables during the experiments. Each experiment lasted for at least 45 hours with alternating refreshing (5min) and measuring intervals (23 min). The length of each measuring interval was set to 23 min in order to have a significant decrease in oxygen concentration, but also the highest possible data resolution. During refreshing intervals oxygen-saturated water from the surrounding tank was pumped into the respirometer chamber to re-establish the oxygen saturation to 95-100%. After a short delay (3 min) allowing for homogenous mixing in the chamber, the oxygen decline was interpreted as respiration rate. Within each measuring period, the oxygen saturation and the water temperature were measured by a microprocessor oximeter (WTW, Oxi 539) and recorded with a frequency of 1 Hz. Experiments were performed at temperatures of 10, 13, 16 and 19°C and were divided into acclimation periods and routine phases (Figure 3-2) based on procedures described in Herrmann and Enders (2000). Results of the data collected during the routine phase of experiments (Figure 3-2) were previously published (Meskendahl *et al.* 2010). During these phases fish activity was relatively constant, so that no clear trends were detectable between respiration and activity. In contrast, during the acclimation period the decreasing oxygen consumption rates were associated with decreasing swimming speeds and turning rates (Figure 3-2).This corroborated the assumption that the elevated metabolism during this period was mainly caused by elevated swimming activities of the fish. Technical problems with the image recording program induced a reduction in the image acquisition rate for some of the conducted experiments after 13-20 hours. We therefore decided to use only values from the first 7-28 hours of each experiment for further analysis depending on the beginning of the routine phase (and less variable measurements), the behaviour of fish and availability of image sequences captured with a constant frame rate of 4Hz.

Since sprat is a highly sensitive fish susceptible to scale loss, it was not possible to select only individuals of the same body mass before experiments. Thus, fish had mean wet weights ranging from 6.90 to 9.76 g and a mean total length of 10.6 cm (± 0.3). After each experiment fish were removed and rapidly killed by an overdose of anaesthetic (MS-222 at >0.2 g l^{-1}). The respirometer was closed again without cleaning and oxygen uptake was measured for at least 24 h to determine bacterial respiration. For each temperature one value for bacterial respiration was calculated as average value and subtracted from the total oxygen consumption rate of the preceding experiments (Meskendahl *et al.* 2010).

3.2.3 Image acquisition

Fish were filmed under constant infra-red light conditions using a CCD-Camera (Ecoline TV7002, Security Center) above the respirometer and an illumination box covered with a light reflecting foil and infra-red LEDs below the respirometer-chamber (Figure 3-1). This design provided a bright and constant background against which the fish appeared as dark objects in the images. The grey value images (Figure 3-1) had a size of 320x240 pixel and were recorded on a PC in a separate room with a frequency of 4 Hz using the program i-Corder® (V2T VISION TO TECHNOLOGY GmbH, Germany).

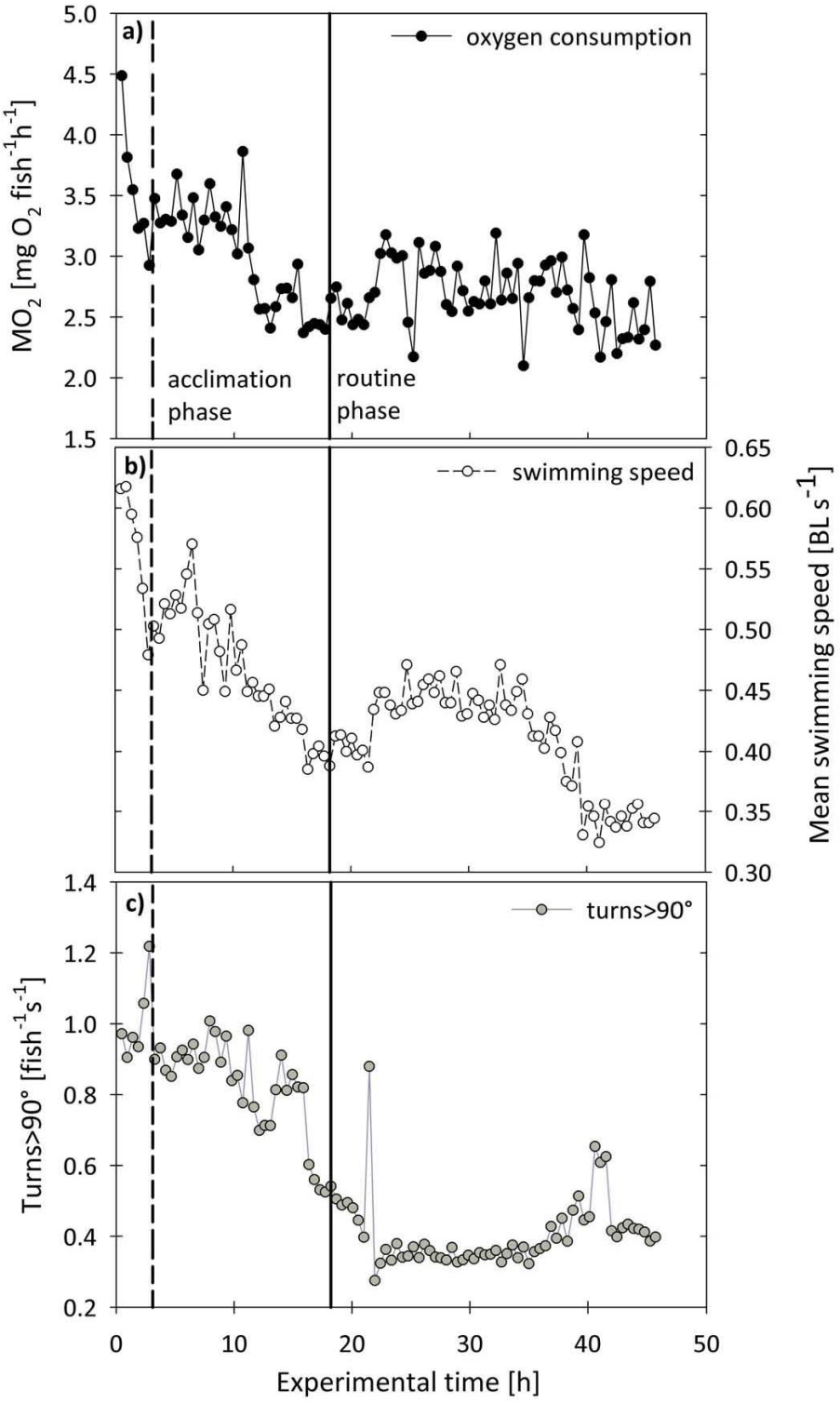

Figure 3-2 Oxygen consumption (**a**; mgO_2 $fish^{-1}$ h^{-1}) of sprat at 19 °C over experimental time (h) and the corresponding mean swimming speeds (**b**; body length s^{-1}) and turns >90°(**c**; $fish^{-1}s^{-1}$). The vertical solid line indicates the beginning of the routine phase; the dashed line separates the initial period including possible effects of handling stress on MO_2. For analysis of activity related metabolic rates of sprat only values from the acclimation phase after exclusion of the first 2-6 measurements (initial period) were used. The separation procedure between routine and acclimation phase followed the description in Herrmann and Enders (2000)

3.2.4 Automated fish tracking

To analyse the movements of the fish, a program (hereafter: fish-tracker) running with MATLAB® (Version 7.3.0.267,The MathWorks Inc., Image-Processing-Toolbox Version 5.3, Database-Toolbox Version 3.2) was designed, which recognized fish movements as changes of fish positions from picture to picture and saved the x- y- positions of each fish in each frame in a database (MySQL). With a graphical user interface (gui) it was possible to visualize the fish center locations on the actual image, along with the tracking history from the previous twelve frames (Figure 3-3).The processing of the fish-tracker is explained with the following steps:

1) Object identification and allocation: All Objects (fish and other particles) were identified by their grey values, which were higher than a predefined threshold value representing the background field. Fish were separated from other particles by a size threshold (number of pixels forming the object) and labelled with an identification-number (ID). The original grey value image was then converted into a binary picture, with identified fish being coded as one, whereas the background and non-fish pixels were coded as zero. The geometric centre (centroid) of the pixels representing a fish was calculated by the “first moment” method, which is a measure of the distribution of the area of a shape in relation to an axis.
2) Estimation of expected fish positions: A Kalman-Filter (Kalman 1960) was used to estimate the expected positions of each fish on the following image. The Kalman filter is a method of combining noisy (and possibly missing) measurements and predictions of the state of an object to achieve an estimate of its true current state. This estimation filter was based on the detected positions of each fish in the last five to twelve images. The Kalman filter was extended by a nearest neighbour method in order to allow for multiple object tracking and to avoid any correspondence problems. This method determines the prospective position of all identified fish based on the individual positions of the previous image: For each object the nearest neighbour (Euclidean distance) with regard to the calculated positions from the Kalman filter was identified. The two objects from the current and the previous frame were assumed to be identical if there was a clear minimum distance and this distance was below a predefined threshold value. All positions of fish not clearly identified run through a further process: 1. If two fish were swimming directly upon each other, the ID for one of them was removed in this frame. In the following frame the nearest object to the crossing location received a new ID. 2. If a fish was not clearly identified in the last five images it`s ID was removed from these five frames, but the same fish could be recognized in the subsequent frame as a new object with a new ID number.
3) Handling of overlapping objects: If objects are very close to each other, correspondence might be incorrect and cause the nearest neighbour method to fail. Therefore overlapping objects needed to be separated by another procedure: When

two or more fish were overlapping, so that the surface area of this object exceeded the maximum area of an individual fish, these objects were separated by morphological erosion and dilation (Haralick *et al.* 1987; Gonzales & Woods 2004). When two fish were swimming directly one after another they formed an object, which exceeded the maximum length of an individual fish. These two fish were then separated along the axis of the second moment. This means that the positions and directions of the two fish were determined by a segmentation of the oversized object along the major axis of an ellipse, equivalent to the objects, and under consideration of their orientation and mass centre (see "regionprobs" in MATLAB®).

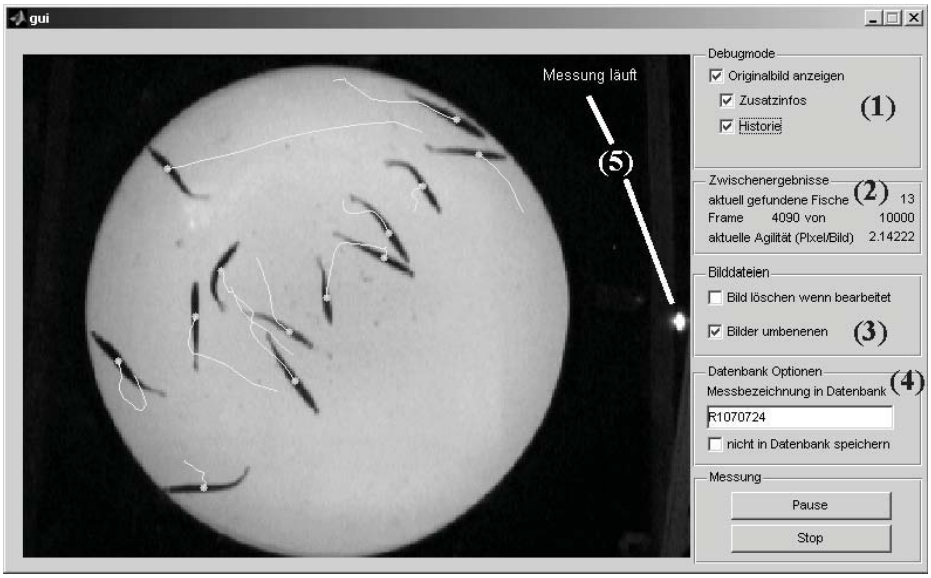

Figure 3-3 Graphical user interface (gui) of the fish-tracker for the automated tracking of sprat in an intermittent-flow respirometer. During the processing it was possible to show the original images with the option to display the centre points of each fish and the tracking history of the last twelve images as lines (1). Information on the actual tracked image (2) was given below. Image processing options (3) and database options (4) had to be adjusted before the start of the program. The IR-LED was powered only during measuring phases (5)

3.2.5 Determination of swimming speeds and direction changes

To translate the position data obtained from the fish-tracker in absolute swimming speeds of the fish, the velocity of flow in the respirometer chamber had to be considered. The velocity pattern was analysed with a small neutrally buoyant plastic ball (drifter) which was floating freely in the water column. Its drift path was monitored with a frequency of 4 Hz and positions were analysed by the fish-tracker. Drift-experiments were undertaken at six different flow-through rates ranging from 1.62 to 4.50 l min^{-1}. There were no indications of turbulences induced by the current based on the observations of swimming fish and the drifter experiments, nor were fish showing any behaviour indicating stability problems.

A second program was designed (in MATLAB®), which analysed the position-data of the drift-experiments to develop a velocity model. The mean water velocity was calculated from all obtained x- and y-positions of the drifter and the respective time steps. The estimated velocity field had an elliptic form due to the unidirectional inflow of water in the chamber and the flow rate increased from the middle of the tank within the ellipse and decreased at the edge of the chamber (Figure 3-4). Mean calculated drift speeds ranged from 0.16 to 4.97 cm s^{-1}, depending on the position in the tank. The model was used to estimate the water velocity at each x-y-position at a given mean flow rate through the chamber.The resulting value was then added to the swimming speed of a fish at the same position, calculated from the x-y-positions from two consecutive images.

The swimming direction of a fish was determined from the difference of two positions between two successive images assuming that fish moved only forward. The change in swimming direction of each individual fish was then calculated as the difference of swimming directions between two successive images. Mean swimming speeds (BL s^{-1}; cm s^{-1}) and direction changes (°$fish^{-1}$ between consecutive images) were calculated for each experimental interval from the x-y-Positions of the fish and the derived estimates of their swimming directions. During a sharp turn (90-180°) a fish turned more or less on the same place, so that the covered distance during a sharp turn was negligible and swimming could be defined as any forward movement of a fish recognized on at least five consecutive images. Swimming speeds and turning rates were averaged per fish group and measuring interval (23 min) in order to relate swimming activities to mean oxygen consumption rates (MO_2) of the respective measuring interval. The coefficient of variation (CV, %) of mean swimming speeds and the number of turning rates with different angles was used to estimate variability in activity within experiments.

3.2.6 Calculation of oxygen consumption rates

Taking into account the chamber volume and the displaced volume of the fish, the actual oxygen uptake rate in one measuring interval in mgO_2 h^{-1} of the fish group was calculated by linear regression:

$$V_{O2} = C_t (V_c - V_f) \quad (1)$$

V_{O2} = oxygen consumption (mgO_2 h^{-1}); C_t = mean decrease of the oxygen concentration in the respirometer (mgO_2 l^{-1} h^{-1}); V_c = chamber volume (ml); V_f = fish volume (ml). Only oxygen consumption rates with a regression coefficient >0.9 times the average of all regression coefficients (~ 0.97) were selected to calculate metabolic rates. The total oxygen uptake was then divided by numbers of fish and fish wet weight after values for bacterial respiration were subtracted.

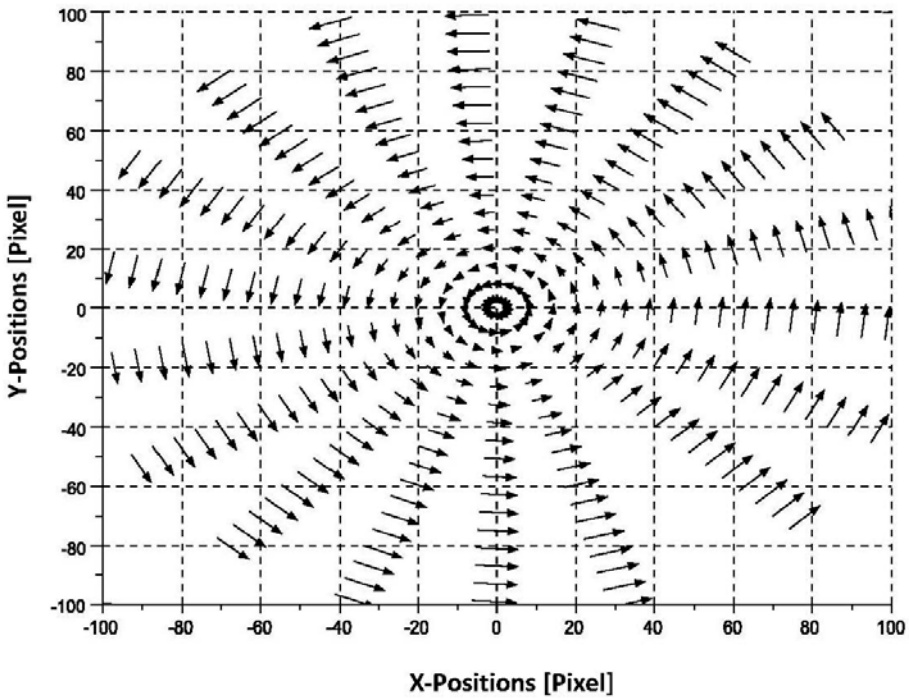

Figure 3-4 Vector graphic representing the velocity field within the respirometer chamber. The model was used to estimate for the water velocity at each position in the tank. Velocities are represented by arrows at some selected positions in order to illustrate the overall picture. For each flow-through rate used in experiments a reference number was added so that the specific velocities for this flow-rate could be determined. The resulting value for the water velocity (positive or negative) was then added to the swimming speed values of the fish. Average speeds ranged from 0.16 cm s^{-1} in the centre to 4.97 cm s^{-1} at the edge of the chamber

3.2.7 Data selection

The first 2-6 measuring intervals (1-3 hours) of all experiments were excluded from further analysis (see Figure 3-2) as in these phases oxygen consumption of the fish group was extremely high due to handling stress and possible effects of elevated levels of stress hormones. Many fish manoeuvred in close proximity to the tank walls during these intervals. This stress-related swimming behaviour was not adequately represented by mean swimming speeds or turning manoeuvres. During our experiments most individuals calmed down and showed normal swimming behaviour approximately three hours after the introduction of fish into the respirometer chamber. The occurrence of normal swimming (when no fish were swimming close to the walls) was controlled by inspection of the respective images in the periods up to four hours after experimental start.

3.2.8 Model selection

The relationship between metabolic rate and temperature in fish is generally described by an exponential relationship (Fry 1957). The temperature coefficient for the standard metabolic term *c1* was adopted from Meskendahl *et al.* (2010) for some of the tested models in order to reduce the number of parameters to be estimated. Oxygen consumption is related to body mass by a power function (Ware 1978) in accordance with formulas used in bioenergetics models (*e.g.* Rudstam 1988; Megrey *et al.* 2007). However, the weight range in the present study was not adequate for determining metabolic scaling effects (Table 3-1) and we therefore used the previously estimated metabolic scaling exponent for standard metabolism of k = 1.073 from Meskendahl *et al.* (2010), where different fish sizes were used. The relationship between swimming speed (U) and metabolic rate was modelled as a power function (Videler and Nolet 1990; Ohlberger *et al.* 2005, 2006), which is based on hydrodynamic principles and allows for species comparisons when standard metabolic rates are assumed to be different (Korsmeyer *et al.* 2002; Papadopoulos 2008). The application of a power function resulted in a higher explained variance (r^2) than the application of an exponential model when fitted to the complete data set. We tested different models considering the temperature dependency of swimming and turning costs, respectively (Table 3-2). We found that costs for swimming in relation to water temperature were best described by an exponential function of temperature in terms of explained variance (r^2) and parameter significance ($p<0.05$). The data of the present study were not sufficient to estimate any body mass effects on swimming speeds as they were obtained from fish with only slightly different body masses (Table 3-1). We therefore used swimming speeds in body length per second (BL s^{-1}) to relate fish activity to metabolic rate instead of cm s^{-1}, because fish swimming speed varies with fish body mass (Ware 1978) and therefore with total fish length. The energy available for moving is always related to the muscle mass, which itself is a function of size (Schmidt-Nielsen 1972). This means that a small fish spends more energy in relation to its body size than a larger conspecific for swimming with the same speed in cm s^{-1}.

In addition to mean swimming speeds, turning rates (Krohn and Boisclair 1994) or turns with high angles (Enders and Herrmann 2003) may account for a substantial part of the metabolic costs during spontaneous activity (Krohn and Boisclair 1994). We therefore calculated the turning rates (°s^{-1}) for each tracked fish per experimental interval. Mean turning rates per interval were in the range between 87 and 194 °s^{-1} (mean = 135% ± 20) and were not directly correlated (linear regression, r^2 >0.2) with the observed oxygen consumption rates or mean swimming speeds, respectively. The observed turning rates were therefore divided into three categories: 1) turns with less than 90°s^{-1}, 2) turns between 90 and 180°s^{-1} and 3) turns larger than 180°s^{-1}. Overall, turning rates with small turning angles (<90°) were observed much less frequently than sharper turning movements. The proportion of turns<90° per experimental interval was between 4-39% (mean = 17%). Turning rates with higher angles (90-180°s^{-1}) accounted for 27-79% (mean = 64% ± 8) of all turning movements

and turns with more than 180°s^{-1} accounted for 1-52% (mean = 17% ± 9) of total turning movements. Thus, most turns were conducted with angles higher than 90°s^{-1}. As turning with small angles (<90°s^{-1}) were performed less frequently and were to some extent included in mean swimming speeds, we tested the number of turns >180° ($fish^{-1}s^{-1}$) and number of turns >90° ($fish^{-1}s^{-1}$) for explaining variability in oxygen consumption rates in addition to mean swimming speeds in our modelling approach. Such movements with high turning angles are known to account for a significant part of spontaneous activity metabolism (Enders and Herrmann 2003). Simple linear regression for the single data sets (per experiment) resulted in a higher regression coefficient (r^2) when oxygen consumption was modelled as a function of turns>90°, than with other estimates of manoeuvrings. Thus, we included only turns>90° in our modelling approach for the complete data set. Although accelerations of single fish could be determined from the data of tracked fish, mean values for the fish groups were always close to zero and were not correlated with mean MO_2. However, according to findings by Webb (1991) some rectilinear accelerations and decelerations are also represented by frequent sharp turns.

Based on these assumptions different regression models were developed (Table 3-2) to determine the relationship between metabolic rates of sprat, water temperature, fish weight and swimming characteristics using a stepwise non-linear regression analysis (nonlinear least-squares, R development Core Team 2011, package = “stats”) with a data set (*n*) of 240 metabolic rate values with the corresponding activity measurements. These values were obtained from 10 experiments at four different temperatures with a total of 104 fish (Table 3-1). Models (Table 3-2) were compared using the following criteria: an overall regression coefficient r^2 >0.5, significance of parameter estimates and lower values for Akaike information criterion (AIC). Model validation included tests for variance homogeneity (residual plot) and normally distributed measurement errors (Shapiro-Wilk-test) following the recommendations by Ritz and Streibig (2008).

3.3 Results

3.3.1 Swimming characteristics

Within each experiment, mean swimming speeds and the number of turns>90° per interval varied over time and resulted in variable oxygen consumption rates (Figure 3-2). Mean swimming speeds during experiments ranged from 0.28 to 0.74 BL s^{-1} (Table 3-1) and the coefficient of variation (CV; %) in swimming speed during the acclimation phase was similar for all temperatures ranging from 15.8% at 19°C to 19.0% at 10°C. Only for experiment No. 3 at 13°C variability in swimming speed was much lower (2.7%). These results indicate that spontaneous swimming was generally variable during the acclimation phases of experiments (Figure 3-2), but not different among the different tested temperatures (Table 3-1). The mean number of turns>90° per interval was also variable between experiments with values ranging from 0.414 to 2.321 $fish^{-1}s^{-1}$ and the lowest CV of

6.8% in experiment No. 3 and highest CV of 20.8% in experiment No. 10. Thus, the number of turns was also highly variable within experiments, but there was no trend with water temperature. An individual fish was able to perform one turning movement of ~90° with maximal bending of its body within 0.25 s or two turns within 0.75 s, but also turns of ~ 180° within one second (Figure 3-5). This is also reflected by the high mean number of turns>90° of up to 2.3 $fish^{-1}s^{-1}$ for one experimental interval (Table 3-1). Distances between neighbouring individuals were in the range from 0.57-2.77 total body length.

Table 3-1 Metabolic rates (MO_2, mgO_2 $fish^{-1}h^{-1}$) of sprat with different mean wet weights (WW, g) at different temperatures (T, °C) and the range of related measurements of swimming speed (U, BL s^{-1}) and the number of direction changes >90° (M, $fish^{-1}h^{-1}$)

Exp No	N of fish	Mean WW	T [°C]	MO_2 ± SD [mgO_2 $fish^{-1}h^{-1}$]	Range of U		Range of M	
					Min [BL s^{-1}]	Max [BL s^{-1}]	Min [$fish^{-1}s^{-1}$]	Max [$fish^{-1}s^{-1}$]
1	11	8.80	10	2.088 ± 0.357	0.46	0.72	0.839	1.376
2	11	9.39	10	2.792 ± 0.291	0.49	0.71	1.026	1.886
3	10	7.82	13	2.430 ± 0.220	0.47	0.52	1.402	1.735
4	12	7.06	16	2.416 ± 0.364	0.28	0.55	0.997	2.321
5	10	7.93	16	2.316 ± 0.209	0.50	0.58	0.822	1.132
6	13	8.35	16	2.513 ± 0.217	0.58	0.67	0.701	1.013
7	8	8.35	16	2.787 ± 0.244	0.61	0.74	0.414	0.572
8	12	9.76	16	3.533 ± 0.221	0.49	0.65	1.023	1.271
9	9	9.51	19	3.053 ± 0.434	0.40	0.64	0.852	1.218
10	8	6.90	19	3.549 ± 0.779	0.53	0.73	0.525	1.487

Exp No = Experiment number; N = number; SD = standard deviation;
Min = minimum value; Max = maximum value; BL = total body length in cm; 1 BL ~ 10.6 cm

3.3.2 Spontaneous swimming costs

Mean oxygen consumption per experiment ranged from 2.088 mgO_2 $fish^{-1}$ h^{-1} at 10°C to 3.549 mgO_2 $fish^{-1}$ h^{-1} at 19°C (Table 3-1). The coefficient of variation was lowest for experiment No. 8 with 6.3% and highest for experiment No. 10 with 19.1%. The relationship between oxygen consumption and the number of turns for the single data sets was best described by a simple linear function (higher r^2), whereas the relationship between oxygen consumption rate and swimming speed was best described by a power function for the single data sets. For the complete dataset of 240 metabolic rate measurements and the corresponding swimming speeds and numbers of turns>90° eight different models (Table 3-2) were tested to describe the relationship between the metabolic rate (MO_2, mgO_2 $fish^{-1}h^{-1}$) of sprat and mean swimming speed (U, BL s^{-1}), number of turns (M, $fish^{-1}s^{-1}$), body mass (WW; g) and water temperature (T, °C). All tested models were based on the same data set from 10 experiments (see Table 3-2 for model comparisons). The experimental design from the present investigation with the relatively high variability in activity did not allow for a sufficient estimation of the standard metabolism (first term in equation 2) in relation to the different tested temperatures and available fish body weights. Therefore in a number of models (Table 3-2), the weight exponent *k* and temperature exponent *c1* were fixed to the

values estimated from the routine phase data (Meskendahl *et al.* 2010). Finally, the following equation was found to be the best prediction of a metabolic rate during spontaneous activity in sprat (r^2 = 0.683; Table 3-2):

$$MO_2 = 0.032\ WW^{1.073}\ e^{0.078\,T} + 0.533\ U^{3.281}\ e^{0.139T} + 0.859\ M \quad (2)$$

Here, the weight exponent of k = 1.073 and the temperature exponent of c_1 = 0.078 within the first term were fixed to the values obtained by Meskendahl *et al.* (2010). The application of this model shows, that metabolic rates showed a greater increase with swimming speed at 19°C than at 10°C. At zero turning metabolic costs are 32% lower than with the inclusion of turns at the mean value of 1.07 turns ($fish^{-1}s^{-1}$). However, this model slightly overestimates the lowest values and underestimates the metabolic rates at the upper end of the measurements (Figure 3-6 a). The highest rates were all obtained from the same experiment (No. 10; Table 3-1) with the highest variability in swimming speed and at the highest tested temperature of 19 °C. For model validation residuals were plotted versus fitted values (Figure 3-6 b) and the explanatory variables: numbers of turns (Figure 3-6 c) and swimming speeds (Figure 3-6 d). There are no patterns or trends in residuals for the fitted values and residuals were normally distributed (Shapiro-Wilk test, W =0.98, $p<0.05$), which justifies the selected model in this instance. For the swimming speed residuals no heteroscedastic trends are obvious, but residuals for the number of turns ($fish^{-1}s^{-1}$; Figure 3-6 c) indicate that the model slightly overestimates the metabolic costs at the lowest measured oxygen consumption rates, which were all obtained from experiment No. 7 (Table 3-1). However, excluding experiment No. 7 and No. 10 from the analysis revealed a non-significant estimation for the temperature exponent for swimming speed c (Model 5 in Table 3-2) and a lower r^2 value of 0.674. We additionally tested to what extent the oxygen consumption rates were not explained by temperature effects using the equation $MO_2 = a\ WW^{b}\ e^{c\,T}$ with b = 1.073 from Meskendahl *et al.* (2010). The resulting parameter estimates were a = 0.134 and c = 0.047 (nonlinear least-squares, R development Core Team 2011, package = "stats"). Fitting the observed values against the fitted values revealed that only 15.9% of the variance in oxygen consumption was explained by water temperature and body mass effects.

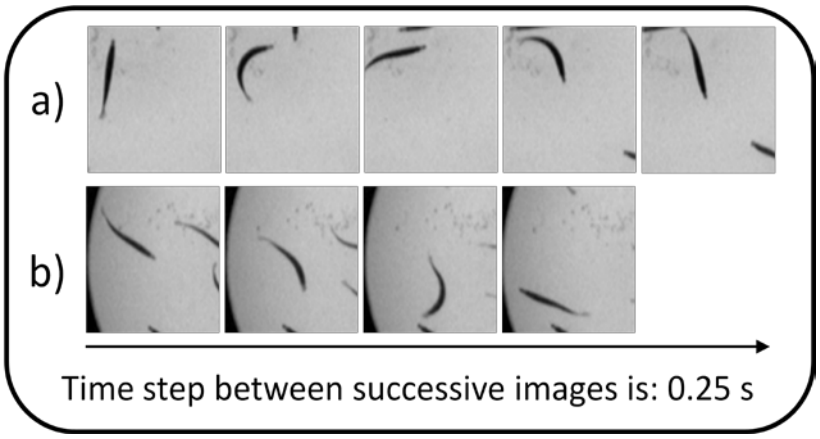

Figure 3-5 Example of typical turning movements of an individual sprat during respirometry experiments. In the upper sequence (a) one fish shows two turns directly one after another within 1.75 s and in the second sequence (b) one turn of ~180° was performed within less than one second

Table 3-2 Predictive models for the metabolic rates (MO_2; mg O_2 $fish^{-1}$ h^{-1}) of sprat using wet weight (WW, g), temperature (T, °C); mean swimming speed (U, BL s^{-1}) and the number of turns >90° (M, $fish^{-1}h^{-1}$) as explicative variables

Model	Model formula	*p*	Df	r^2	RSS	AIC	NS
1	$MO_2 = a\ WW^{1.073} e^{(c\ T)} + b\ U^{v} + d\ M$	5	235	0.701	31.191	203	a
2	$MO_2 = a\ WW^{1.073} e^{(0.078\ T)} + b\ U^{v} + d\ M$	4	236	0.548	47.053	300	
3	$MO_2 = a\ WW^{1.073} e^{(0.078\ T)} + b\ U^{v} e^{(0.078\ T)} + d\ M$	4	236	0.665	34.910	228	
4	**$MO_2 = a\ WW^{1.073} e^{(0.078\ T)} + b\ U^{v} e^{(c\ T)} + d\ M$**	**5**	**235**	**0.683**	**33.129**	**216**	
5	$MO_2 = a\ WW^{1.073} e^{(c\ T)} + b\ U^{v} e^{(c\ T)} + d\ M$	5	235	0.679	33.265	219	
6	$MO_2 = a\ WW^{1.073} e^{(c1\ T)} + b\ U^{v} e^{(c2\ T)} + d\ M$	6	234	0.725	28.634	184	c1,b
7	$MO_2 = a\ WW^{1.073} e^{(0.078\ T)} + b\ U^{v} e^{(c\ T)} + d\ M\ e^{(c\ T)}$	5	235	0.663	35.166	232	
8	$MO_2 = a\ WW^{1.073} e^{(0.078\ T)} + b\ U^{v} e^{(c1\ T)} + d\ M\ e^{(c2\ T)}$	6	234	0.706	30.670	201	c2

p = number of parameters; Df = degrees of freedom; r^2 = coefficient of determination; RSS = residual sum of squares; AIC = Akaike information criterion; NS = non-significant parameter estimates

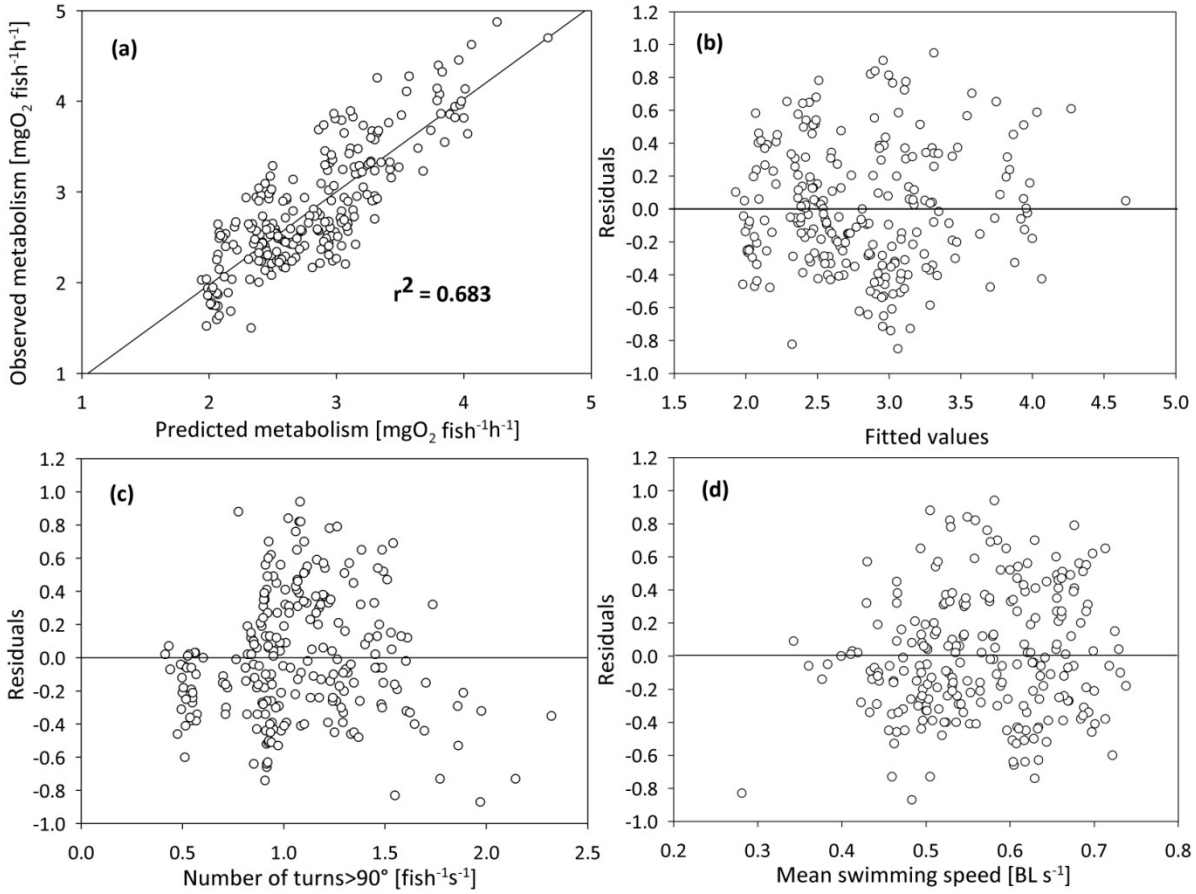

Figure 3-6 Observed metabolic rates (mg O_2 $fish^{-1}$ h^{-1}) versus predicted values from model 4 (Table 2) with the corresponding coefficient of determination (r^2;**a**) and residuals from the model versus fitted values (**b**), residuals for the number of turns>90° ($fish^{-1}$ s^{-1}; **c**) and mean swimming speeds (BL s^{-1}; **d**). Parameter estimates for the nonlinear regression model are presented in Table 3-3

Table 3-3 Parameter estimates and corresponding 95%-confidence intervals (CI) of the non-linear regression analysis $MO_2 = a\ WW^{1.073} \exp^{(0.078\ T)} + b\ U^{v} \exp^{(c\ T)} + d\ M$, relating the metabolic rates (MO_2; mgO_2 $fish^{-1}$ h^{-1}) of sprat to temperature (T, °C), body mass (WW, g), swimming speed (U, BL s^{-1}) and turns>90° (M, $fish^{-1}s^{-1}$)

Parameter	Estimated value ± standard error	95%-CI lower level	upper level	r^2
a	0.032 ± 0.004	0.023	0.041	
c	0.139 ± 0.019	0.102	0.181	
b	0.533 ± 0.137	0.248	0.782	0.682
v	3.281 ± 0.697	1.937	4.706	
d	0.859 ± 0.055	0.754	0.969	

r^2 = coefficient of determination

3.4 Discussion

The main goal of our experiments was to determine the metabolic costs of sprat during spontaneous activities including relationships with the number of sharp turns (>90°) and mean swimming speeds. This was mainly precipitated by the need for basic input parameters for bioenergetics modelling. We further wanted to test whether spontaneous activities differ in terms of energy costs from previous measurements of straight-line swimming clupeoids.

3.4.1 Fish behaviour and tank effects

Shortly after the introduction of fish into the respirometer chamber, some individuals were swimming in close proximity to the tanks walls. Such apparent abnormal swimming behaviour is assumed to be induced by stress when fish are confined in small tanks or after handling (Brett 1964; Brett and Sutherland 1965). However, this behaviour occurred only in the first 1-3 hours after the transfer into the chamber. Therefore, experimental phases with such behaviours were excluded from further analysis. Handling stress in fishes can in general result in catecholamine stimulation and the subsequent release of cortisol (Wendelaar Bonga 1997). This can increase cardiac output, oxygen uptake and the mobilisation of energy substrates, but it is still unclear how long elevated levels of cortisol or other stress hormones are sustained (Wendelaar Bonga 1997) and to what extent this is not reflected in increased activity. Davis and Schreck (1997) found for juvenile coho salmon, that elevation in oxygen consumption rates was largely eliminated within 1 h after handling stress. Since fish in our experiments were pre-acclimated to the smaller tank size and experimental temperatures, stress was only induced by the careful transfer into the respirometer. Thus, we assumed that fish were no longer stressed when they showed their normal swimming behaviour. The exclusion of the first 1-3 hours has most likely excluded elevated oxygen uptake rates which are not directly reflected in higher swimming activities. The present results indicate that the higher oxygen consumption rates in the beginning of experiments could be mainly explained with higher swimming speeds and turns>90° (see also Figure 3-2).

The observed low swimming speeds (0.28 - 0.74 BL s^{-1}) and high numbers of turns (0.414 - 2.321 $fish^{-1}s^{-1}$) in the respirometer were probably influenced by the artificial confinement of the fish. The tank was relatively small due to restrictions imposed by respirometry techniques (Steffensen *et al.* 1989) and this can affect the swimming characteristics of fish (Tang and Boisclair 1993; Steinhausen *et al.* 2010). However, we found a clear relationship between MO_2 and turns>90°, so that it is now possible to estimate the energy costs associated with such manoeuvrings. In nature sprat shows most likely variable, spontaneous swimming movements as they are not always swimming in polarized schools, but also swim in random patterns, *e.g.* under low light conditions during the night (Shvestov *et al.* 1983; Nilsson *et al.* 2003) or during feeding where sprat shows frequent sharp turns (pers.obs.). However, the final application of our model results to field situations requires

quantifications of the frequency of such movements under natural conditions, *e.g.* by underwater video observations of sprat in the shallow regions of the Baltic Sea.

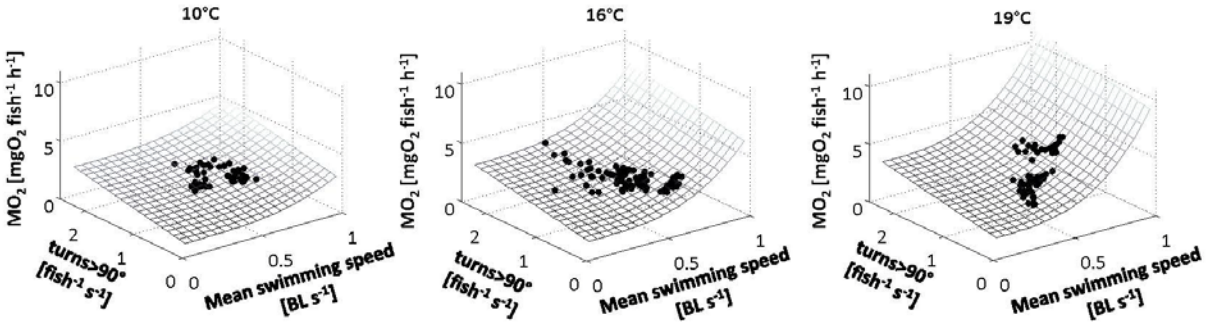

Figure 3-7 Metabolic rates (MO_2; mg O_2 $fish^1$ h^{-1}) of sprat (8.5 g WW) in relation to mean swimming speed (BL s^{-1}) and turning rates (turns>90°; $fish^{-1}$ s^{-1}) for different water temperatures (°C) determined with the model output (grid) and the corresponding weight-corrected original values (black dots). Parameter estimates and statistics of the regression model are provided in Table 3-3. Data for 13°C are not shown as there were only data from one experiment available

3.4.2 Classification of spontaneous active metabolic rates

Metabolic rates of sprat in the present study were clearly higher than standard or routine metabolic rates which were obtained during the routine phase of experiments (Figure 3-2; see also Meskendahl *et al.* 2010). The spontaneous activity metabolism of sprat at the mean swimming speed of 0.57 BL s^{-1} and the mean number of turns>90° of 1.096 $fish^{-1}$ s^{-1} is 1.21-1.36-times the costs associated with standard metabolism (Meskendahl *et al.* 2010) at temperatures of 10°C and 19°C, respectively. This is in line with previous calculations by Sirois and Boisclair (1995) for "spontaneous activity metabolism" of brook trout (*Salvelinus fontinalis*), which was 1.0-1.4-times the costs associated with standard metabolism. Although Sirois and Boisclair (1995) introduced the term "spontaneous activity metabolism" and several investigations focused on energy costs of spontaneously active fish (*e.g.* Boisclair and Tang 1993, Krohn and Boisclair 1994; Tang *et al.* 2000; Tudorache *et al.* 2009), there is still no generally accepted term for this metabolic level. This makes an explicit classification of the presently measured metabolic rate difficult. However, spontaneous swimming in fish was defined as a swimming mode involving both steady and unsteady components (Videler 1993; Tudorache *et al.* 2009), whereas the latter includes turning manoeuvres, accelerations and decelerations (Blake 1983; Videler 1993). Tudorache *et al.* (2009) determined the energy costs of spontaneously swimming surfperch (*Embiotoca lateralis*) under consideration of different turning angles, speeds, accelerations, decelerations and pectoral fin beat frequencies. They found relatively low swimming speeds and high degrees of manoeuvring during spontaneous swimming of surfperch, similar to the results of the present study (Table 3-1 and 3-4). In conclusion, the metabolism of sprat from the present study reflects mainly an elevated routine metabolic rate under spontaneous swimming (with sharp turning movements) and could be termed "spontaneous activity

metabolism". This metabolic rate should be clearly distinguished from the previously measured metabolic rates during the routine phase and under less variable swimming activities (Meskendahl *et al.* 2010).

3.4.3 Metabolic costs for swimming and direction changes

The selected model (No 4, Table 3-1) describing the relationship between MO_2 and swimming characteristics resulted in a swimming speed exponent of v = 3.281 ± 0.697. Thereby v represents the steepness of the increase of MO_2 with increasing activities (Blake 1991) and can be directly used for comparisons among species (Korsmeyer *et al.* 2002). This exponent was slightly higher than the majority of swimming speed exponents in power functions described for straight-line swimming fishes, ranging from 1.1-3.0 (Videler and Nolet 1990). However, these values were all obtained from species not directly related to sprat. In most studies on clupeoids exponential functions were used to describe the relationship between swimming speed and metabolism (*e.g.* Boggs 1991; van der Lingen 1995; Macy *et al.* 1999). Exponential functions relating MO_2 to swimming speed are basically useful for the extrapolation of metabolism to zero swimming speed in order to get estimates on standard metabolism (Papadopoulos 2008), but are inadequate for direct comparisons among species when standard metabolic rates are different (Korsmeyer *et al.* 2002). This makes direct comparisons of swimming costs among cluepoid fishes difficult. We therefore determined the increase in oxygen consumption resulting from a doubling in swimming speed within the measured range of the respective study (Table 3-4). Thereby, MO_2 of sprat increased 2.15-fold with an increase in swimming speed from 0.4-0.8 BL s^{-1}, which is only slightly higher than factors found for other clupeoids derived from experiments at various speeds, ranging from 1.70-fold elevation in MO_2 for *Engraulis mordax* (Boggs 1991) to 1.96-fold for the pilchard *Sardinops sagax* (van der Lingen 1995). The observed mean swimming speed of sprat with 0.57 BL s^{-1} was somewhat lower than the mean routine swimming speed reported for other clupeoids of 1.32 BL s^{-1}(Table 3-4), but relatively close to that of *S. sagax* (van der Lingen 1995) where also similar metabolic rates were measured.

Table 3-4 Comparison of the increase in metabolic rate (MR) with a doubling in swimming speed (U) of sprat from the present study with that in related fishes during routine swimming, considering the range of tested speeds in the respective studies. T = temperature; WW = wet weight; TL = total length; * = fork length

Species	Reference	Swimming mode	T [°C]	WW [g]	TL [cm]	MR at U [$mgO_2\ g^{-1}h^{-1}$]	Mean U [BL s^{-1}]	Range of U	Factor
S. sprattus	present study	spontaneous	16	8.5	10.6	0.220	0.57	0.4-0.8	2.15
Sardinops sagax	van der Lingen 1995	voluntary; constant	16	146	25.6	0.178	0.78	0.5-1.0	1.96
Engraulis encrasicolus	James and Probyn 1989	voluntary	16	6.3	8.8	0.087	1.82	1.0-2.0	1.63
Brevoortia tyrannus	Macy *et al.* 1995	forced; constant	20	283	25.5	0.197	1.62	1.0-2.0	1.65
Engraulis mordax	Boggs 1991	forced; constant	17	8.7	8.1	0.264	1.07	1.0-2.0	1.70

To the best of our knowledge, there are no comparable measurements of metabolic costs for high turning rates or direction changes like those from the present investigation for related species nor is there any information on the frequency of such movements in sprat or herring under natural conditions. However, sprat show such movements primarily during feeding when snatching single prey items. We observed for feeding sprat in the laboratory that biting events (*e.g.* when feeding on *Artemia salina*) occur primarily during upward swimming. At the upper end of the prey patch sprat show at least one sharp turn, followed by a downward swimming burst (Brachvogel *et al.* 2012.). Thus, the energy costs of turns>90° are of particular interest to assess the costs for feeding in sprat. With our final model it is now possible to estimate the energy costs of sharp turns, but one has to keep in mind that we measured metabolic rates of entirely fasted fish. Metabolism will increase during feeding due to digestive processes (SDA-effects). Thus, we cannot directly relate the turning costs to the energy consumed during feeding. The final application of our results to field situations requires further information on prey densities in patches, spontaneous swimming patterns of sprat during feeding and measurements on SDA-effects.

3.4.4 Temperature effects

Active metabolic rates of fish generally depend on water temperatures (Fry 1971), but there is no general pattern to describe the relationship between swimming costs and temperature for all species. On the one hand, swimming speed during straight-line swimming increases with increasing temperatures between 8 and 20°C in perch, *Perca fluviatilis*, and roach, *Rutilus rutilus* in non-respirometry experiments (Linløkken *et al.* 2010), but also in *S. sagax* over temperatures from 10-22°C in a swimming respirometer (van der Lingen 1995). Positive correlations between constant speeds and temperature are assumed to occur only below certain water temperatures (Colby 1973; Schaefer 1986; Klumb *et al.* 2003). Thus, in bioenergetics models for alewife, *Alosa pseudoharengus* (Stewart and Binkowski 1986), and herring, *C. harengus* (Rudstam 1988), swimming speed is modelled as a function of temperature only below 9°C and it is assumed that at higher temperatures swimming speeds remain constant. In the present study sprat showed similar swimming speeds during spontaneous activities for all tested temperatures (10-19°C) ranging from 0.28 to 0.74 BL s^{-1} over all experiments (Table 3-1), but total metabolic costs showed a greater increase with increasing temperature (Figure 3-7). Thus, metabolic costs were best described as exponential function of temperature within the swimming speed term (Table 3-2). The relationship between oxygen consumption and turning rates was, however, not affected by temperature and was therefore modelled without a separate temperature term (Eq. 2, Table 3-2). However, as other authors assumed that swimming speeds will decrease below 9°C (*e.g.* Stewart and Binkowski 1986; Rudstam 1988), we cannot conclude whether swimming speeds in sprat are constant at lower temperatures than investigated in the present study.

On the basis of our best-fit model (Table 3-2) it is possible to obtain different Q_{10} values. The first one is based on the temperature exponent for standard metabolism of c =

0.078 (Q_{10} = 2.18) adopted from Meskendahl *et al.* (2010) and reflects the temperature dependency of R_S. The second value can be estimated from the temperature exponent for swimming speed (*c* = 0.139) with Q_{10} = 4.0 and predicts the higher energy costs for swimming at higher temperatures at a constant speed. An overall Q_{10} value for temperature dependency of MO_2 can be calculated by the application of our final model (Table 3-2) for different swimming speeds and turning rates. Assuming a swimming speed of 0.5 BL s^{-1} and turns >90° of 0.5 $fish^{-1}h^{-1}$, results in an overall Q_{10} value for MO_2 of 2.1 over the temperature range from 9-19°C for 8.5 g sprat. At a higher swimming speed of 0.8 BL s^{-1}, and again turning rates of 0.5 $fish^{-1}s^{-1}$, the overall Q_{10} would be 2.8 for the same temperature range and fish size. Such a high Q_{10} of metabolic rates plays a role in defining the temperature niche for optimal growth, especially if the Q_{10} of maximum consumption rate is lower, as indicated from unpublished results from the authors.

3.5 Acknowledgements

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4 Manuscript 3: Functional responses of juvenile herring and sprat in relation to different prey types

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Abstract

The relationship between particulate-feeding rates and prey concentrations (functional response) of juvenile herring and sprat (5-9 cm total length) was investigated in controlled feeding experiments monitored by an underwater camera system. A special tank system was developed allowing the regulation and quantification of low prey concentrations (1-160 L^{-1}). Non-evasive *Artemia* nauplii was used as prey to estimate the maximum biting rate of both predators. In contrast, *A. tonsa* with high escape ability was used as a realistic prey type. Herring and sprat showed a type II functional response for both prey types. Nonlinear mixed effects model revealed no significant difference between the functional responses of both predators, except that herring showed significantly higher biting rates than sprat at *A. tonsa* concentrations below ~40 L^{-1}. For both predators feeding rates were significantly higher with *Artemia* nauplii than with *A. tonsa*. Video analysis indicated that sprat, unlike herring, is an obligate particulate-feeder.

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4.1 Introduction

Planktivorous fish play a key role in the pelagic ecosystem as they have a marked impact upon their prey communities and are an important source of food for piscivorous predators (Rudstam *et al.* 1994). The intermediate trophic level - often occupied by one or few small pelagic schooling species – can exert a major control on whole ecosystems, namely in upwelling regions (Cury *et al.* 2000) and in the Baltic Sea with sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) as dominant small pelagic fish (Möllmann *et al.* 2004). Information on the relationship between sprat and herring per capita feeding rates and prey concentrations (functional response; Holling 1959, 1966) is therefore of particular interest to understand their top-down control of the zooplankton community.

Functional response curves are essential components of predator-prey models (Jeschke *et al.* 2002) and can potentially determine the stability of predator-prey dynamics (Sarnelle and Wilson 2008). There are three main functional response models (Holling 1959, 1966) which differ in the way feeding rates depend on prey concentrations. The type I functional response takes a linear form, yielding a constant predation risk for the prey. This type is typical for filter feeding organisms either having a negligibly small handling time or being able to search and capture prey while handling other food (Jeschke *et al.* 2004). Clupeoid fish like herring (Gibson and Ezzi 1985), alewife *Alosa pseudoharengus* (Janssen 1976), Cape anchovy *Engraulis encrasicolus* (James and Findlay 1989), California anchovy *Engraulis mordax* (Leong and O'Connell 1969) and sardine *Sardinops sagax* (van der Lingen 1994) exhibit both filter- and particulate feeding modes. They generally filter-feed at high concentrations of small particles and particulate-feed at low concentrations or on larger prey (Gibson and Ezzi 1985; Lazzaro 1987). Thus, at low prey concentrations particulate-feeding herring and sprat are assumed to show a type II or type III functional response as prey handling time is higher than during filter-feeding. In a type II response, the number of prey killed per time increases with increasing prey density but the rate of increase is progressively reduced until an asymptote is reached at high densities (Juliano 2001). This type of functional response can potentially destabilize prey populations because predation risk increases with deceasing prey population density (Sarnelle and Wilson 2008). In contrast the type III response is characterized by a sigmoid shape leading to a decreasing predation risk at low prey densities (Sarnelle and Wilson 2008). The difference between both types lies in the behaviour of predators at low prey densities: the type III model assumes that predators are inefficient at finding prey if prey concentration is low or that there is a threshold level below which predators do not respond (Shin *et al.* 2010). The type II functional response has been implemented likewise in process models investigating single species bioenergetics and prey encounter (Stockwell and Johnson 1997; 1999; Varpe and Fiksen 2010) and in marine ecosystem models like NEMURO (Megrey *et al.* 2007) and ATLANTIS (Fulton *et al.* 2004) to model fish and plankton population interactions. However, only few data exist to validate this assumption (type II) and to set the actual model parameters. Previous research on the

feeding behaviour of herring was mainly focused on factors triggering particulate- and filter-feeding, like prey size, food density and light level (Gibson and Ezzi 1985, 1990, 1992; Batty *et al.* 1990). For this purpose, Gibson and Ezzi (1985) examined the feeding behaviour in herring mainly at high prey concentrations of up to 1000 L^{-1}. However, the abundance of the primary prey source of sprat and herring, calanoid copepods, can be highly variable in space and time with values below 10 L^{-1} (Colebrook 1979; Broekhuizen and McKenzie 1995) to more than 100 L^{-1} in areas of higher aggregation (Soeteart and van Rijsijk 1993; Folt and Burns 1999). Since low prey concentrations were not tested before, the actual form of functional response remained unknown. Our study intends to fill this gap, with an investigation of feeding rates of juvenile sprat and herring at lower prey concentrations (1 to 160 L^{-1}) where the difference between type II and III functional responses should be detectable.

Juvenile herring and sprat form mixed species schools in the coastal waters of the Baltic and North Seas and are therefore strongly associated with each other (De Silva 1973; Arrhenius and Hansson 1993; Maes and Ollevier 2002). Both fish species mainly feed on calanoid copepods, whereas larger herring (> 15-20 cm) also consume larger prey like mysids, amphipods, polychaetes, decapods and fish eggs (Last 1987; Casini *at al.* 2004). Therefore, food competition, particularly among the 0-group, could be a relevant factor if food resources are limited. We hypothesize that juvenile herring reach higher feeding rates than sprat at the same prey concentrations, based on findings from field stomach data analysed by Maes and Ollevier (2002).

Feeding experiments were conducted with two different prey types differing in their escape ability. Non-evasive *Artemia salina* nauplii as prey allowed the estimation of the potential maximum biting rate of sprat and herring. Results with *Artemia* nauplii are assumed to reflect the functional response of sprat and herring feeding on non-evasive prey items such as cladocerans (Viitasalo *et al.* 2001), which represent a large part of the diet of both predators (De Silva 1973; Arrhenius 1996). In contrast adult *Acartia tonsa* have a well-developed escape response (Singarajah 1969; Trager *et al.* 1994; Kiørboe 2010) and were therefore used to represent the typical copepod diet of sprat and herring (Maes *et al.* 2005). We hypothesize that the feeding rates of herring and sprat are higher with *Artemia* nauplii than with *A. tonsa* due to the shorter handling time needed for a non-evasive prey.

The major objectives of this study were therefore (1) to identify and parameterize the functional response models of sprat and herring and (2) to compare the functional responses of juvenile sprat and herring to identify possible competitive advantages, and (3) to test the effect of escape behaviour of different prey types on the handling time of both predators.

4.2 Materials and Methods

4.2.1 Capture and maintenance of experimental fish

Young-of-the-year (YOY) herring were caught in June 2009 with a hand-operated dip-net (area: 4 m^2; mesh size 6 mm) in the Harbour of List, Sylt (North Sea, 55°1'11N; 8°36'8 E). YOY sprat were captured in July 2009 and 2010 in the Harbour of Wendtorf (Baltic Sea, 54° 41' N; 10° 3'E). Fish were transported in a 700 L box with aerated seawater to the aquarium facilities of the Institute of Hydrobiology and Fisheries Sciences at the University of Hamburg. Prior to experiments fish were maintained in large groups of 100-500 individuals in circular tanks (1000 L) supplied with continuous flow of mechanically and biologically filtered, artificial seawater (Aqua medic) from the recirculation system. Sprat and herring were kept at an ambient temperature of 16.0 ± 0.1°C (mean ± SD) and at salinities of 16 and 32 PSU, respectively. Fish were maintained under a 13 L:11 D light regime and were fed an artificial pellet diet (*Marico advance 0.5-0.8 mm*, Coppens International bv) and live *Artemia salina* nauplii (*SEPArt-Cysts*, INVE Aquaculture) twice a day. Both herring and sprat were acclimated to laboratory conditions for 2 months prior to the onset of the experiments.

Table 4-1 Summary of feeding experiments with herring and sprat

Herring			**Sprat**		
exp. series	number of exps.	prey concentration [L^{-1}] at 50-60 min interval per exp.	exp. series	number of exps.	prey concentration [L^{-1}] at 50-60 min interval per exp.
Artemia nauplii as prey					
H1	5	16; 16; 10; 40; 67	S1	3	156; 59; 26
H2	5	19; 16; 21; 55; 79	S2	5	14; 8; 70; 66; 32
H3	5	32; 41; 16; 8; 56	S3	5	42; 68; 6; 17; 144
H4	2	55; 43	S4	2	50; 78
			S5	2	73; 27
			S6	2	30; 78
A. tonsa as prey					
H4	3	18, 8; 15	S4	2	44; 104
H5	4	41; 31; 16; 26	S5	2	26; 73
			S6	2	26; 97

Within each experimental series 2-5 experiments with different initial prey concentrations of lived *Artemia* nauplii or *A. tonsa* were generated randomly. Nomenclature of experimental series based on: fish species (H = herring; S = sprat) and the different fish groups (numeration), which each consisted of 10 fishes. During an experiment, only data from the 50-60 min interval onwards was used for the statistical analysis, therefore, prey concentration at this interval was considered as starting point

4.2.2 Prey types

Non-evasive *Artemia salina* nauplii (771 ± 90 µm total length, N = 316; 0.00171 ± 0.00038 mg dry weight; N = 151) were used in experiments as a slow moving prey species with low escape responses in order to determine the maximum feeding rate of the fish. Contrary, late copepodites (C5) and adults of *Acartia tonsa* (690 ± 75 µm prosoma length, N

= 180; 0.00207 ± 0.00091 mg dry weight, N = 50) were used as natural prey organism with a well developed escape response (Singarajah 1969; Buskey 1994; Kiørboe 2010). Copepods were cultured within 240 L tanks at a salinity of 18 PSU and at temperatures between 20-22°C following the procedures described in Holste and Peck (2006). To ensure a better comparability of feeding rates obtained for different prey types, we used similar sized *Artemia* nauplii and *A. tonsa*. Average body lengths (µm) of the prey items from each experiment were measured using ImagePro Plus® on digital images captured with a camera (Leica-300®) mounted on a binocular microscope (Leica®) at a magnification of 50. As *Artemia* nauplii are generally reddish in colour, we fed *A. tonsa* with *Rhodomonas sp.* shortly before each experiment to assure a similar coloration of both prey types. *A. tonsa* swim in a sink-and-hop pattern, whereas *Artemia* have a smoother swimming behaviour. By comparing prey of similar size and pigmentation, it is assumed that prey with active and irregular swimming behaviour is more attractive for predators than prey with reduced motion (Buskey *et al.* 1993). However, we assumed this aspect can be neglected if light intensity in the water clearly exceeds the threshold for particulate-feeding (0.01 lx for herring; Batty *et al.* 1990) and if the offered prey is relatively large.

4.2.3 Experimental setup

Overall we used 60 sprat (63.0 ± 5.3 mm total length) and 50 herring (81.7 ± 7.1 mm total length) in 49 experiments (Table 4-1). The experiments were conducted using groups of fishes since it is not possible to maintain species like herring and sprat individually. Two to five experiments with different initial target prey concentrations from approx. 10 to 160 L^{-1} were performed per experimental series (Table 4-1). During an experiment the initial target prey concentration was maintained constant for 60 min by adding food continuously. This period with constant prey concentrations ensured that prey items were distributed homogeneously within the experimental tank, and that fish acclimatized to the food supply. Furthermore the data from this period were used to test for any trends due to saturation or feeding stimulation. After this period food addition was stopped and prey concentration decreased exponentially due to feeding activity of fish and the water overflow (Figure 4-1). A series of experiments was carried out with the same fish group of 10 individuals within one week feeding on either *A. tonsa* or *Artemia* nauplii (herring = 5 fish groups; sprat = 6 fish groups; Table 4-1). At the start of each series fish were carefully transferred into the experimental tank and therein acclimated for 2 days. During this period no food was supplied. Unfortunately, experiments with *A. tonsa* were performed less frequently than with *Artemia* nauplii due to limits in the production of live copepods given the longer generation time compared to *Artemia* (Table 4-1).

The experimental tank (401 L; Figure 4-1) was placed in a separate room of the laboratory to prevent external disturbance of fish during experimentation. The equipment used for food supply and the determination of prey concentrations was visually separated from the tank by a wall. Additionally, the inside of the tank was covered with black foil to

minimize the stress of fish. The experimental tank was divided into a prey-mixing chamber (162 L) and a fish chamber (239 L) separated by a PVC-panel with two circular holes (Figure 4-1) allowing water exchange between the two chambers. A gentle circular water drift of double filtered (20 and 1 µm pore diameter) artificial seawater from the recirculation system was powered by the aeration (Figure 4-1). That water flow promoted a homogeneous distribution of prey items within both chambers. Through the upper hole prey was transported into the fish chamber and through the lower hole uneaten organisms streamed back into the mixing chamber (Figure 4-1). To create lighting conditions of 1.3-1.0 lx, allowing particulate-feeding (Batty *et al.* 1990), a 40-watt bulb was placed above the tank. Feeding behaviour of fish during experiments was recorded using an infrared camera (TV 7143 *ABUS*; Resolution: 420-600 TV-Line) mounted underwater near the bottom of the tank in the PVC-panel (Figure 4-1). A light-reflecting box with infrared-LEDs allowed the observation of fish as dark objects against an illuminated background (Figure 4-1). Each chamber of the experimental tank contained a tube (I + II; Figure 4-1) for food addition to achieve a specific initial target prey concentration in the whole tank (Figure 4-1). This concentration was maintained constant for 60 min by continuously supplying (peristaltic pump; 36 mL min^{-1}; Gilson Minipuls II) a prey suspension of a known concentration into the mixing chamber (Figure 4-1). The necessary concentration of the prey suspension was calculated from the theoretical losses of food due to fish's feeding and the water overflow. The assessment of prey loss due to fish's feeding was initially based on biting rates observed from preliminary experiments. The additional observations from each experiment were then used to constantly update the estimation of prey loss. Experiments lasted until no feeding fish were observed on the screen. The total duration of an experiment was 90-150 min. To determine the actual prey concentrations in the tank during the entire experiment all prey items lost through the overflow (3.83 L min^{-1}) were collected every 10 min (time intervals = 0-10, 10-20, 20-30…) by a 100-µm mesh-bottomed cup (Figure 4-1). Collected prey items of all time intervals were counted under a binocular for all concentrations < 25 L^{-1}, and for every second time interval at concentration higher than 25 L^{-1}. If prey was not counted manually, their numbers were estimated by dry weight using weights from counted probes as a reference.

4.2.4 Data analyses

All statistical analyses were performed using the R statistical program version 2.13.1 (R Development Core Team 2011). The prey concentration (C_t) for a 10 min time interval was calculated from $C_t = N_t / FR$, where N_t is the total number of prey items (N) in the collection-cup divided by the length of the time interval, and FR is the overflow rate (3.83 L min^{-1}). The recorded videos (25 frames s^{-1}) were played with half-speed to determine the average biting rate for each 10 min time interval of an experiment. In each 10 min time interval 15-20 individual fish were tracked. Fish were selected randomly by pointing with eyes closed on the computer screen and taking the nearest fish that was well in focus. Each individual fish

was tracked for 10-60 s and its biting rate (biting acts s^{-1}) was determined visually. It was considered that only one prey item was consumed per biting act.

Trends in biting rates over the first 60 min of constant prey concentrations could indicate saturation effects, physical fatigue or a stimulation of feeding activity. Thus, we compared the biting rates of the 10-20 and 50-60 min time intervals for the highest initial target prey concentration of each exp. series (Table 4-1) by a student's paired *t*-test.

We also determined the duration between two biting acts on a frame-by-frame basis as total feeding time t_t. Total feeding time is composed of different activities: search (t_s), detection (t_d), approach (t_a) and prey handling (t_h). Handling time for *Artemia nauplii* and *A. tonsa* was defined as the time for prey biting: t_h is the time between opening and closing of the fish mouth. For *A. tonsa* handling time additionally included the time for *S*-shaped curvation of the body before biting. Unfortunately, it was not possible to quantify t_s, t_d and t_a separately due to the insufficient resolution of the infrared camera and the fast succession of these events. However, under the assumption that t_h was constant per prey type, we derived the relationship between the sum of t_s, t_d and t_a and prey concentration. For better comparability of total feeding times for both prey types we only used data of sprat exp. series S4, S5 and S6, as they experienced similar prey concentrations of *Artemia* nauplii and *A. tonsa* (Table 4-1). Prey specific comparisons of total feeding times on an exp. series basis were done by a student's paired *t*-test.

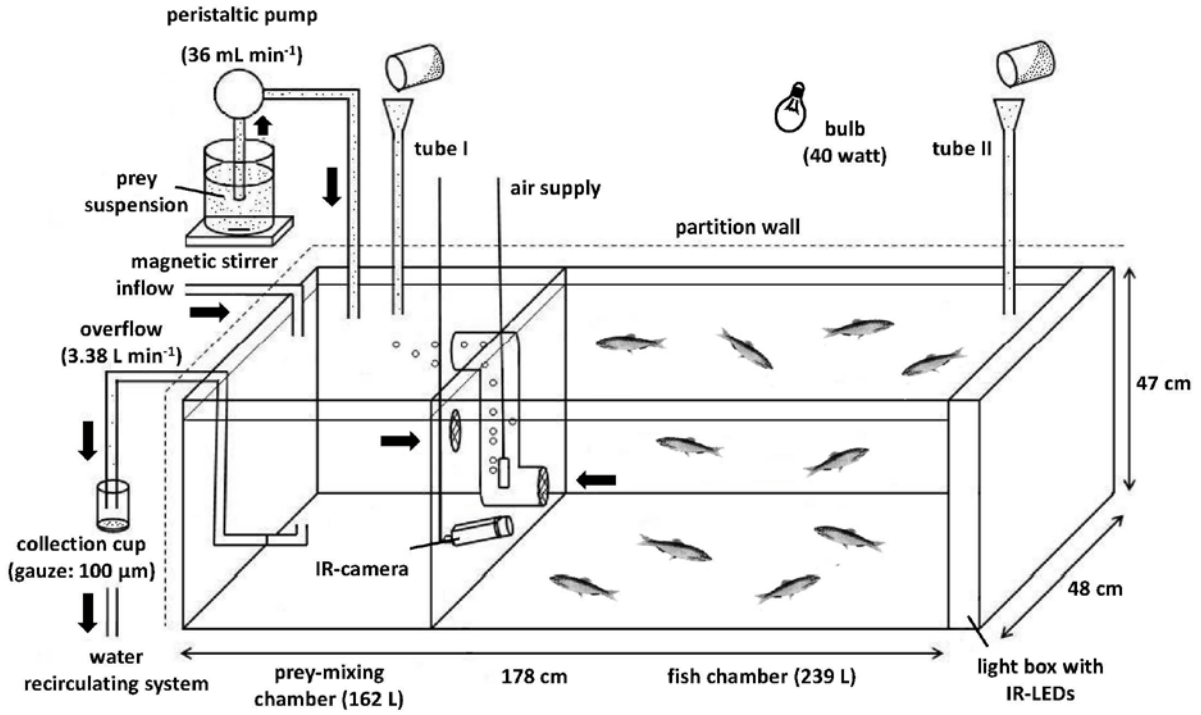

Figure 4-1 Diagram of experimental arrangement. All needed apparatus were visually separated from the tank by a partition wall (dotted line)

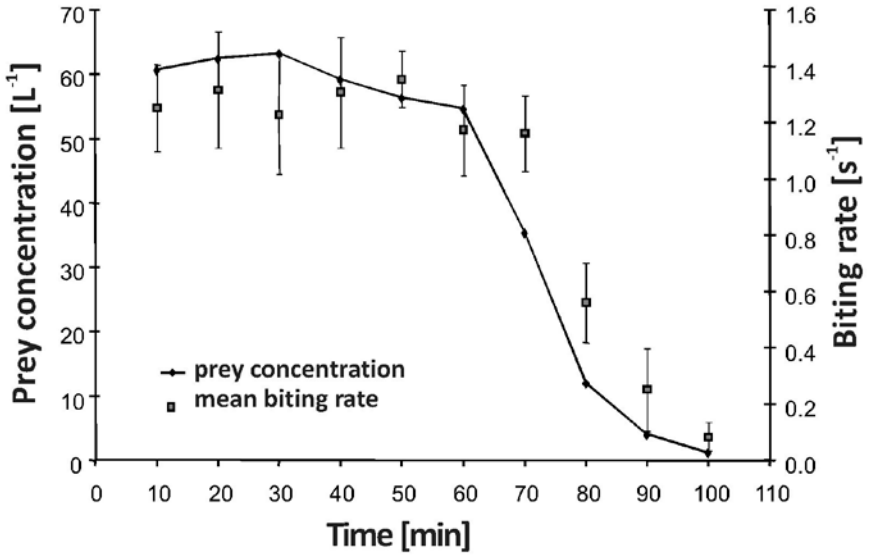

Figure 4-2 Example of change in prey concentrations (L^{-1}) and mean biting rates (s^{-1}) (± SD) over experimental time for herring feeding on *Artemia* nauplii (Table 4-1, H2, prey concentration at 50-60 min interval = 55 L^{-1}). Prey items were constantly added during the first 60 min in order to maintain a stable concentration. After this period food addition was stopped and prey concentration decreased exponentially due to feeding activity of fish and the water overflow

4.2.4.1 Model fitting

We analysed the feeding responses of sprat and herring in relation to different prey types (*Artemia nauplii* and *A. tonsa)* and concentrations using nonlinear mixed effects models. The analysis was performed with the nlme-package (version 3.1-101) in R (Pinheiro *et al.* 2011) and followed the descriptions in Pinheiro and Bates (2000) and Zuur *et al.* (2009). Nonlinear mixed effects models were chosen as they can accommodate unbalanced data (prey concentrations) as well as repeated measurements on the same exp. series (Table 4-1). In addition, the models allow for the inclusion of random factors, which account for the between-series variability and heterogeneous variance (Lindstrom and Bates 1990; Pinheiro and Bates 2000; Aggrey 2009). To avoid pseudo-replication only measurements from the 50-60 min intervals onwards were used. Since none of the data series displayed any sigmoid pattern a Michaelis-Menten-model, which is mathematically equivalent to Holling's disc equation model (1959), was used to represent the expected biting rate (*BR*) as a function of prey concentration (*c*):

$$BR_{ij} = BRmax_i \times c_{ij} / (k_i + c_{ij}) + \varepsilon_{ij},\ \varepsilon_{ij} \sim N(0, \sigma^2) \quad (1)$$

where BR_{ij} (s^{-1}) is the *j*th observation of biting rate on the *i*th experimental series (i = 1,….,M j = 1,….,m_i); M is the total number of series, and m_i is the total number of observations on the *i*th series ; c_{ij} is the corresponding prey concentration (L^{-1}); $BRmax_i$ is the maximum biting rate (s^{-1}), and k_i is a constant, which indicates the prey concentration at $BRmax_i/2$; ε_{ij} is a normally distributed noise term and σ^2 is the variance for the residuals. The series-specific parameters $BRmax_i$ and k_i are modelled as the sum of two components:

$$\begin{bmatrix} BR\max_i \\ k_i \end{bmatrix} = \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} b_{1i} \\ b_{2i} \end{bmatrix} = \beta + b_i,\ \mathrm{b_i} \sim \mathrm{N}(0, {\sigma_b}^2) \quad (2)$$

where β is a vector of fixed effects parameters common to all exp. series; b_i is a random effects vector associated with series *i*, which represents the deviation of the expt. series parameters from the population average, and σ^2_b is the variance of the random effects (Lindstrom and Bates 1990; Pinheiro and Bates 2000). It is further assumed that observations made on different series are independent and the within-series errors ε_{ij} are independent of the random effects.

We introduced the covariates prey type and fish species in the model to explain the $BRmax_i$ and k_i series-to-series variation (Pinheiro and Bates 2000). Thus the formulations for $BRmax_i$ and k_i were expanded:

$$\begin{aligned} \mathrm{BR\max_i} &= \beta_1 + \gamma_{01}\mathrm{x_{1i}} + \gamma_{02}\mathrm{x_{2i}} + \gamma_{03}\mathrm{x_{1i}x_{2i}} + \mathrm{b_{1i}}, \\ \mathrm{k_i} &= \beta_2 + \gamma_{11}\mathrm{x_{1i}} + \gamma_{12}\mathrm{x_{2i}} + \gamma_{13}\mathrm{x_{1i}x_{2i}} + \mathrm{b_{2i}}, \end{aligned} \quad (3)$$

$$\mathrm{x_{1i}} = \begin{cases} -1, \mathrm{fish_i} = \mathrm{herring}, \\ 1, \mathrm{fish_i} = \mathrm{sprat}, \end{cases} \quad \mathrm{x_{2i}} = \begin{cases} -1, \mathrm{prey_i} = \mathrm{Artemia}, \\ 1, \mathrm{prey_i} = \mathrm{A.tonsa}, \end{cases}$$

where x_{1i} and x_{2i} are binary indicator variables for fish species and prey type; β_1 and β_2 are, respectively, the average maximum biting rate $BRmax_i$ (*Intercept*) and constant k_i (*Intercept*); γ_{01} and γ_{11} represent the fish species main effect on $BRmax_i$ (*BRmax fish*) and k_i (*k fish*); γ_{02} and γ_{12} are, respectively, the prey type main effect on $BRmax_i$ (*BRmax prey*) and k_i (*k prey*); γ_{03} and γ_{13} represent the fish species-prey type interaction effect on $BRmax_i$ (*BRmax fish:prey*) and k_i (*k fish:prey*). Moreover, β_1, β_2 and γ_{01}, γ_{11}, respectively, represent the $BRmax_i$ and k_i for herring and sprat feeding on *Artemia* nauplii; γ_{02}, γ_{12} and γ_{03}, γ_{13}, respectively, represent the $BRmax_i$ and k_i for herring and sprat with *A. tonsa*.

The next step was to optimize the random part of the model. The very high correlation between $BRmax_i$ and k_i suggested that the random effects model was over-parameterized (Pinheiro & Bates 2000). Thus we decided that only $BRmax_i$ needed random effects as there appeared to be more variability in the $BRmax_i$ estimates than for k_i estimates. In addition, initial data analyses showed that residual spread varied per fish species and prey type, and decreased with increasing prey concentrations. To take into account the heterogeneity of variance we compared different variance functions and chose the appropriate structure by Akaike information criteria (AIC) (Zuur *et al.* 2009). The variance structure was finally modelled with the *varPower* variance function (Pinheiro and Bates 2000), which includes the influences of prey type, fish species and prey concentration on residual spread. To test if the random term is really needed we also compared the AIC of the

mixed effects model with an extended nonlinear regression model without random effects (gnls generalized nonlinear least-squares; nlme-package in R).

4.3 Results

4.3.1 Experimental procedure

In all experiments the number of fish feeding was very high (rarely, 1-2 fish did not feed in an experiment). To test if saturation effects occurred during the experimental phases with constant food concentrations, we compared the biting rates of the 10-20 and 50-60 min intervals for the highest initial prey concentration of each exp. series (Table 4-1). Paired *t*-tests revealed no significant increases or decreases of feeding rates between these intervals for all series (Table 4-2). The strong dependence of biting rates on prey concentrations can be seen in Figure 4-2. Prey concentrations remained stable at the first 60 min of experiment, followed by an exponential decrease of prey concentrations and biting rates after the stop of the food supply. The loss of prey items during an experiment was caused mainly by feeding activity of fishes (84 ± 8 %; N = 512 time intervals) and only to a small extent by the overflow (16 ± 8 %; N = 512 time intervals).

4.3.2 Feeding behavior

In the absence of food, herring and sprat swam around continuously in loose schools. The introduction of prey items initiated a feeding response, characterized by an increase of swimming speeds and the onset of particulate-feeding, depending on prey concentration. During feeding, fish did not school but aligned themselves to prey items with frequent turns and changes of direction. The act of biting, the main feeding mode of herring and sprat, was characterized by a rapid opening and closing of the mouth (Figure 4-3 a, b). Handling time for a simple biting act lasted 0.12-0.16 s. Sprat showed only particulate-feeding, whereas herring sometimes switched to filtering or gulping when food concentrations were > 50 L^{-1}. Gulping is an intermediate feeding type between biting and filtering (Gibson and Ezzi, 1990) and lasted 0.20-0.24 s. During each filtering event of herring the mouth was opened wide and the operculum flared. A filtering event lasted on average 0.68 s (Figure 4-3 c). When herring and sprat were feeding on *Artemia* nauplii both fed only by simple biting whereas fish feeding on *A. tonsa* mostly took up a characteristic *S*-shaped curvation of the body prior to the attack (Figure 4-4). Handling time for a biting act with *S*-shaped curvation lasted on average 0.48 ± 0.19 s, N = 27. It appeared that the body of fish was contracted and released like a spring in order to attack a prey item with higher acceleration. The amplitude of the contraction reached a maximum just before the onset of the strike. Overall, sprat and herring attacked their prey predominantly from below (Figure 4-3, 4-4).

Table 4-2 Results of paired *t*-tests to compare the biting rates of the 10-20 and 50-60 min time intervals for the highest initial prey concentration of each exp. series; *n.s.* non significant with $P > 0.05$

Experimental series	*t* value	df	*P*
Artemia nauplii as prey			
H1	0.0764	11	*n.s.*
H2	1.0472	11	*n.s.*
H3	0.7419	11	*n.s.*
H4	-1.8064	16	*n.s.*
S1	1.573	16	*n.s.*
S2	0.9716	16	*n.s.*
S3	-1.0158	12	*n.s.*
S4	1.5475	13	*n.s.*
S5	0.0371	13	*n.s.*
S6	-1.7261	10	*n.s.*
A. tonsa as prey			
H4	-1.1905	15	*n.s.*
H5	-0.6411	14	*n.s.*
S4	1.5894	12	*n.s.*
S5	0.9564	13	*n.s.*
S6	0.3637	13	*n.s.*

At prey concentrations of > 15 L^{-1} both fish species swam in a vertical zigzag pattern, with repeated bites while swimming upwards at an angle of about 35-45° (Figure 4-5). Near the surface fish performed a 180° turn followed by a downward swimming movement. After another turn the next feeding sequence started immediately (Figure 4-5). In the majority of cases fish did not utilize the full height of the tank (47 cm). Mostly they stopped feeding at 5-10 cm before the surface. Between two and twelve attacks were performed in succession before fish swam downwards. The upward and downward swimming sequences lasted about 2.0-2.5 s and 0.5-0.7 s, respectively. At lower concentrations (< 15 L^{-1}) fish changed their swimming behaviour and did not show a vertical movement pattern like described above. Instead fish increasingly searched more or less at the same water depth.

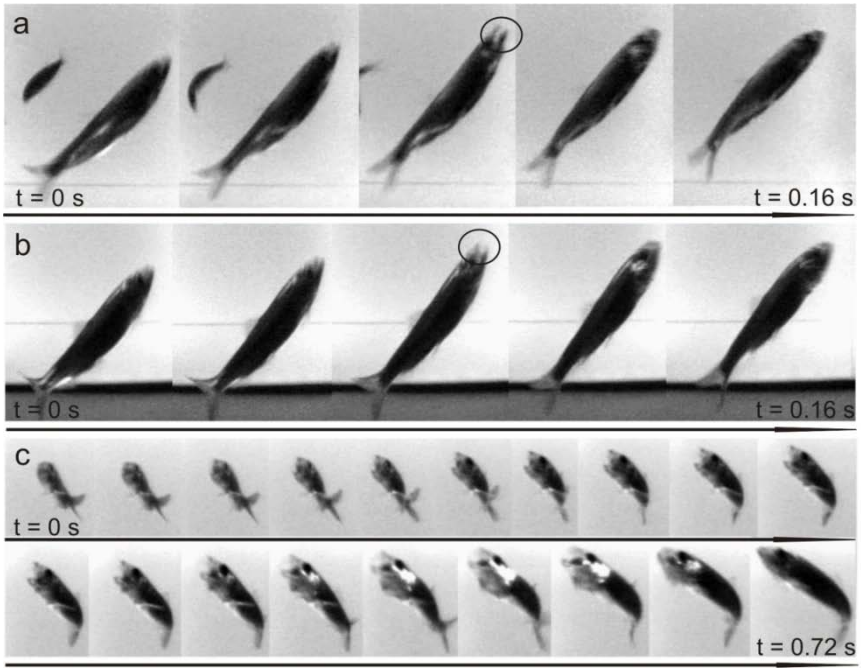

Figure 4-3 Frame-by-frame illustration of typical movements of sprat (**a**) and herring (**b**) during particulate- or filter-feeding (**c**) on *Artemia* nauplii. Circles indicate the moment of mouth opening

4.3.3 Feeding time for *A. tonsa* and *Artemia* nauplii

Frame-by-frame analysis of the feeding behaviour allowed the estimation of total feeding times (s) at different concentrations for both prey types (Figure 4-6). Total feeding time decreased strongly with increasing prey concentration *c* and asymptotically approached a minimum value ($t_{t\ Artemia\ nauplii} = 6.14c^{-0.62}$; $t_{t\ A.\ tonsa} = 6.66c^{-0.49}$; Figure 4-6). The comparison of total feeding times at high prey concentrations (> 20 L^{-1}) for sprat exp. series 4, 5 and 6 (Table 4-1) revealed that sprat showed significantly longer total feeding times with *A. tonsa* (t_{t_min} = 1.08 ± 0.29 s, N = 22) than with *Artemia* nauplii (t_{t_min} = 0.65 ± 0.11 s, N = 17) (paired *t*-test: t_{S4} = -7.906, $P < 0.01$; t_{S5}= -51.669, $P < 0.001$; t_{S6} = -6.450, $P < 0.01$).

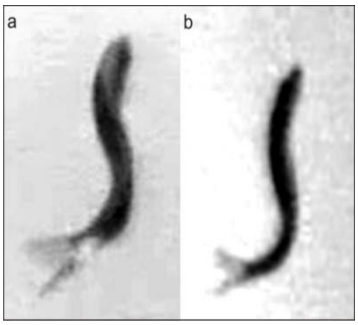

Figure 4 Particulate-feeding with *S*-shape curvation of the body of herring (**a**) and sprat (**b**) feeding on *A. tonsa*

4.3.4 Functional responses

Biting rates of sprat and herring as a function of *A. tonsa* or *Artemia* nauplii concentrations followed in all series a functional response type II (Figure 4-7, 4-8). The mixed effects model described the data considerably better than the extended nonlinear regression model without a random component to account for exp. series (Table 4-1) effects (ΔAIC = 28.55). The *t*-statistics indicated a strong prey-type effect, but no significant fish species effect, with the exception of a significant fish species:prey type interaction on the parameter *k* (p = 0.04) (Table 4-3). This implies that at low prey concentrations (< 40 L^{-1}) the biting rates of herring ($k_{herring:A.\ tonsa}$ = 12.01) with *A. tonsa* were higher than those of sprat ($k_{sprat:\ A.tonsa}$ = 25.42) (Table 4-3). However, the estimated maximum biting rates of herring ($BRmax_{herring:A.\ tonsa}$ = 0.95 s^{-1}) and sprat ($BRmax_{sprat:A.\ tonsa}$ = 1.06 s^{-1}) feeding on *A. tonsa* were similar (Table 4-3). Both predators showed significantly higher biting rates with *Artemia* nauplii than with *A. tonsa* ($p \leq 0.0001$; Table 4-3). The estimated maximum biting rates ($BRmax_{herring:Artemia\ nauplii}$ = 2.17 s^{-1}; $BRmax_{sprat:Artemia\ nauplii}$ = 2.04 s^{-1}) and the parameter *k* ($k_{herring:Artemia\ nauplii}$ = 36.36; $k_{sprat:Artemia\ nauplii}$ = 28.42) with *Artemia* nauplii were not significantly different between herring and sprat. The goodness of the fit of the mixed effects model can be visualized by displaying the fitted and observed values in the same plot (Figure 4-8). Both the population prediction (obtained by setting the random effects to zero) and the within-series predictions (using the estimated random effects) are illustrated (Figure 4-8). Additionally, the coefficient of determination (r^2) from linear regressions of observed versus predicted biting rates were estimated for both fish species and prey types. Values of r^2 for herring and sprat feeding on *A. tonsa* were 0.83 (F = 203.8; $p < 0.001$) and 0.85 (F = 273.4; $p < 0.001$), and on *Artemia* nauplii 0.94 (F = 1161; $p < 0.001$) and 0.95 (F = 2200; $p < 0.001$), respectively. The residuals from the mixed effects model fulfilled the assumptions of normality and homogeneity of

variance. The *varPower* (Pinheiro and Bates 2000) variance function adequately represented the within-series heteroscedasticity.

Table 4-3 Functional response parameters and standard error estimates (± SE) from the nonlinear mixed effect model

Parameter	Estimate ± SE	*t* value	*p*
BRmax (Intercept)	2.173 ± 0.226	9.62	< 0.0001
herring : *Artemia*			
BRmax prey	0.945 ± 0.256	4.829	< 0.0001
herring : *A. tonsa*			
BRmax fish	2.035 ± 0.243	9.051	0.5699
sprat : *Artemia*			
BRmax fish:prey	1.064 ± 0.280	5.257	0.6692
sprat : *A. tonsa*			
k (Intercept)	36.359 ± 5.585	6.511	< 0.0001
herring : *Artemia*			
k prey	12.005 ± 6.011	2.459	0.0001
herring : *A. tonsa*			
k fish	28.421 ± 5.928	5.171	0.1817
sprat : *Artemia*			
k fish:prey	25.416 ± 6.572	4.5	0.0423
sprat : *A. tonsa*			
σ2	0.00156826		
σb2	0.01047601		
logLik	305.293		
AIC	-582.585		
BIC	-532.207		

The model was fitted by restricted maximum likelihood (REML). *BRmax* = maximum biting rate; *k* = Michaelis constant; σ^2 = residual *BR* variance; σ_b^2 = the series variance in *BRmax* within the population; AIC = Akaike information criterion; BIC = Bayesian information criterion; LogLik = log-likelihood. Parameters indicate, on the one hand, the main effect of fish species, prey type and fish:prey interaction on *BRmax* and *k*, and, on the other hand, the estimated *BRmax* and *k* values for sprat or herring feeding on *Artemia* nauplli or *A. tonsa* (listed in Eq. 3)

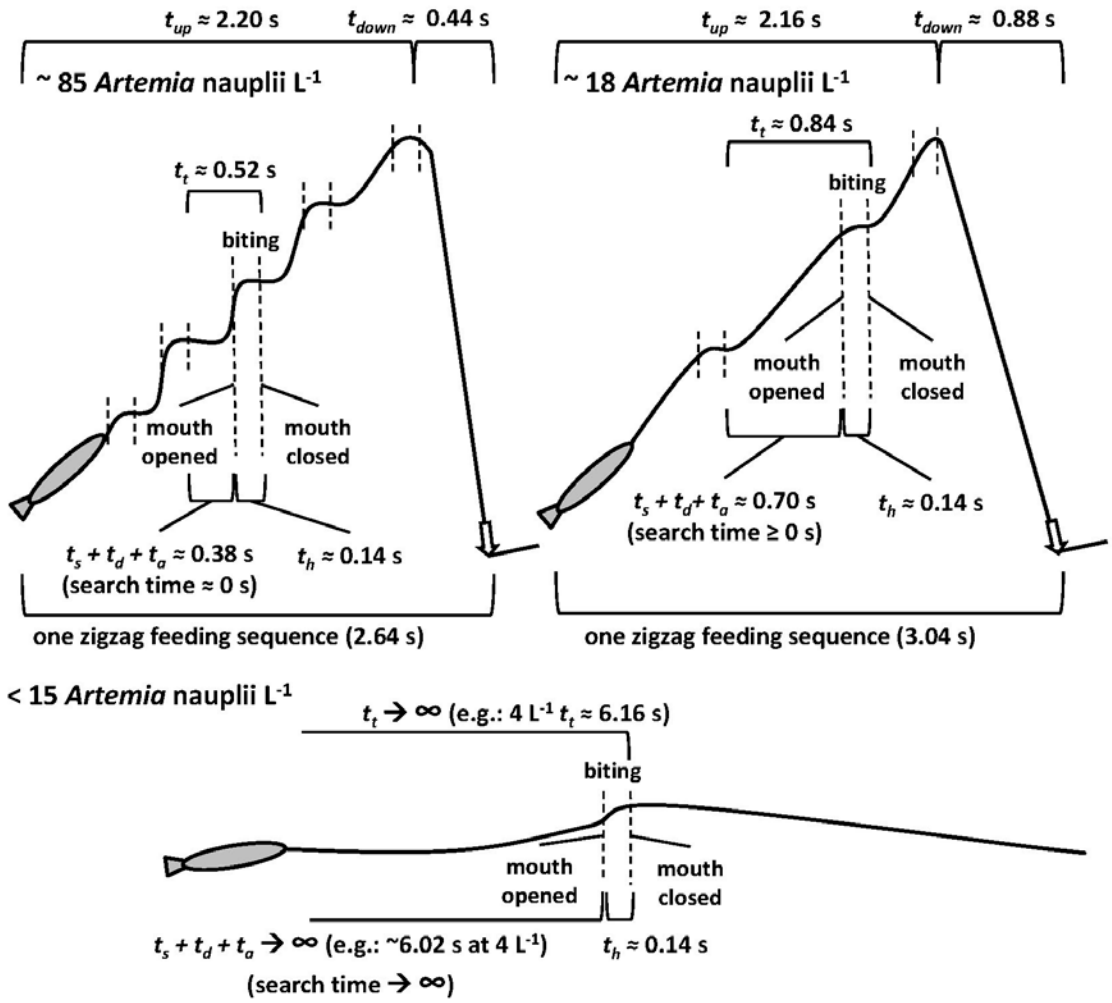

Figure 4-5 Exemplary illustration of the feeding behaviour of sprat (S5, total length = 59.0 ± 4.0 mm) feeding on different *Artemia* nauplii concentrations. t_{up} = time for upward swimming; t_{down} = time for downward swimming; t_t = total feeding time; t_s = search time; t_d = detection time; t_a = approach time; t_h = prey handling time

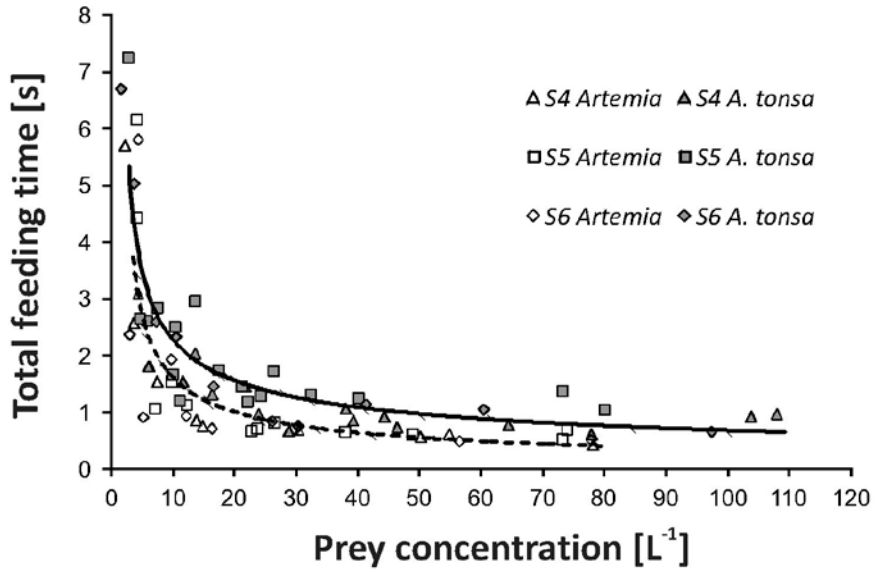

Figure 4-6 Total feeding times t_t (s) (duration between two biting acts) at different prey concentrations c (L^{-1}). Only data from experiments with sprat exp. series S4, S5 and S6 preying on *A. tonsa* (filled symbols and solid line; t_t = 6.66c-0.49; r^2 = 0.77) or *Artemia* nauplii (unfilled symbols and dotted line; t_t = 6.14c-0.62; r^2 = 0.76) were used (Table 4-1)

4.4 Discussion

4.4.1 Experimental procedure

The experimental design enabled the measurement of feeding rates under controlled laboratory conditions using techniques that caused only minimal stress for fish. This is confirmed by the high proportion of feeding fish during the experiments and the high biting rates at high prey concentrations.

To actually observe a fish capturing a copepod, one would need a time resolution of two milliseconds and a spatial resolution of 15 µm in order to see the fish's mouth and the copepod's reaction (Strickler *et al.* 2005). Hence, the underwater images taken in the present study did not directly allow the detection of the feeding success of fish. For the analysis of the functional response type, we had to assume that each counted biting event was successful. This is most likely true when fish were feeding on *Artemia*, but less certain for experiments with *A. tonsa*, which have a relatively high escape responsibility (Singarajah 1969; Buskey 1994; Kiørboe 2010). However, we occasionally observed that fish were starting one feeding attack, but stopped abruptly and began searching for new prey items again. This behaviour most likely reflects an unsuccessful feeding event, where the copepod escaped from the visual field of the fish. We therefore assumed that a fish has successfully ingested one prey item when a biting attack was completed and the opening and closing of the fish's mouth was detectable.

At prey concentrations > 15 L^{-1} sprat and herring showed a vertical zig-zag swimming behaviour (Figure 4-5). The extent of this upwards movement might have been limited by the height of the experimental tank (47 cm). However, fish in our experiments mostly did not utilize the full height of the aquarium. Additionally, *in situ* observations demonstrated that juvenile herring only attacked four to six times in succession while swimming upwards (Kils 1992). This is in line with our results with two to twelve attacks in succession. Thus, the observed feeding behaviour of sprat and herring at the present study is assumed to reflect a normal feeding behaviour.

4.4.2 Feeding behavior

Clupeid fish like herring (Gibson and Ezzi 1985) and anchovy (Leong and O'Connell 1969; James and Findlay 1989) exhibit both filter- and particulate-feeding modes. In the present study herring started to filter-feed at prey concentrations of about > 50 L^{-1}, which is similar to the observations by Gibson and Ezzi (1985) for juvenile herring. In contrast, our results indicated that sprat, unlike other clupeids, is an obligate particulate-feeding species as previously suggested by Bernreuther (2007). Sprat exclusively sticked to particulate-feeding, even at prey concentrations of about 160 L^{-1}. Crowder (1985) suggested that the use of different feeding modes may be dependent on the relation of prey size to predator

size. Durbin (1979) argued that fish particulate-feed when the prey size predator size ratio is in the range between 1:20 and 1:200, whereas filter-feeding occurs when prey size predator size ratio is in the range from 1:150 to 1:20000. He showed that even the juveniles of obligate filter-feeding Atlantic menhaden (*Brevoortia tyrannus*) are actually particulate feeders. Filter feeding is considered to be energetically more expensive than particulate-feeding (Gibson and Ezzi 1992) and thus seems to be beneficial only above a certain predator to prey size ratio and at higher prey concentrations. In the present study juvenile herring (7-9 cm total length) only rarely filter-fed at higher prey concentrations (> 50 L^{-1}), corresponding to an average prey size predator size ratio of 1:100 (prey item ~ 0.08 cm). *In situ* observations by Kils (1992) confirmed likewise that juvenile herring (38 mm mean length) attacked each copepod individually even at high prey concentrations of up to 850 L^{-1}. Clearly distinctive filter-feeding behaviour has only been observed for larger herring (13-20 cm total length; Gibson and Ezzi 1985, 1990, 1992). Contrary to herring sprat stay relatively small (L-infinity = 14.9 cm TL; Alshuth 1989) which probably explains why sprat is an obligate particulate-feeder.

4.4.3 Functional response

The feeding rates of juvenile sprat and herring clearly followed a type II functional response (Holling 1959, 1966) (Figure 4-7). Gibson and Ezzi (1992) and Bernreuther *et al.* (2008) supposed a type II response for herring, but due to the small amount of data at low prey concentrations these results remained uncertain. With our modified experimental design we were able to adjust and monitor feeding rates at very low prey concentrations, where the difference between a type II and a type III functional response would become evident. A type II response implies a high extinction risk for the prey as predation risk per prey capita increases with decreasing prey concentrations. Furthermore, clupeids are able to store large amounts of food items in their gastric cecum, which enables the sustained exploitation of high prey concentrations (Bernreuther *et al*. 2008). Hence, it appears possible that schools of sprat and herring are able to deplete local zooplankton patches within relatively short times given the combination of high maximum biting rates (~ 1 copepod s^{-1}), the high storage capacity and the type II functional response. Thus, we assume that sprat and herring are able to exert strong local top-down effects on prey populations. Hawkins *et al*. (2012) investigated the grazing of sprat schools on zooplankton within an enclosed Lough (Lough Hyne, Ireland). Acoustic surveys indicated that these schools rapidly depleted their surroundings of zooplankton and extensive volumes of water around them were largely devoid of zooplankton. The authors assume that this grazing effect on zooplankton also affected the primary producers with wider ranging implications for the ecosystem (Hawkins *et al.* 2012). Similar cascading effects have been discussed on a larger scale for the Baltic ecosystem as a consequence of the strong increase of the sprat populations in the 1990s (Rudstam *et al.* 1994; Köster *et al.* 2003; Casini *et al.* 2008).

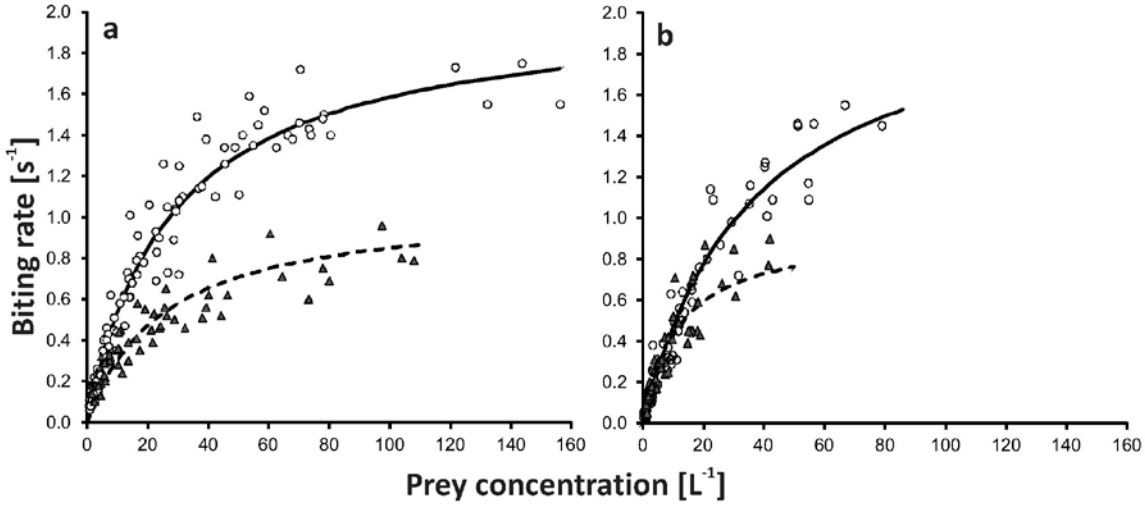

Figure 4-7 Biting rates (*BR*, s^{-1}) of sprat (**a**) and herring (**b**) feeding on *Artemia* nauplii (circles) or *A. tonsa* (triangles) at different concentrations (*c*, L^{-1}). Plotted lines represent functional response type II fitted by the nonlinear mixed effects model *(BR = BRmax* c / (k + c); $BRmax_{spra:Artemia\ nauplii}$* = 2.04 s^{-1}, $k_{sprat:Artemia\ nauplii}$ = 28.42; $BRmax_{sprat:A.\ tonsa}$ = 1.06 s^{-1}, $k_{sprat:A.\ tonsa}$ = 25.42; $BRmax_{herring:Artemia\ nauplii}$ = 2.17 s^{-1}, $k_{herring:Artemia\ nauplii}$ = 36.36; $BRmax_{herring:A.\ tonsa}$ = 0.95 s^{-1}, $k_{herring:A.\ tonsa}$ = 12.01)

A comparison of feeding rates from our study with results of previous investigations on clupeids is difficult due to differences in experimental techniques. Gibson and Ezzi (1992) investigated the feeding behaviour of herring (13-20 cm total length) at much higher prey concentrations of up to ~1000 L^{-1} observing maximum biting rates of about 1.5 s^{-1} and 1.0 s^{-1} in experiments with *Artemia* nauplii and the copepod *Calanus finmarchicus,* respectively. Bernreuther *et al.* (2008) used frozen copepods in experiments with herring (9-13 cm total length) and determined a maximum biting rate of 0.8 s^{-1} at prey concentrations of up to 600 L^{-1}. This rate is surprisingly low due to the non-existent escape response of the frozen food. A possible explanation is a higher proportion of filtering events with increasing prey concentration implying that the visually registered biting events were in fact rather gulping or short filtering events. Another explanation might be that feeding is stimulated more by living food (Buskey *et al.* 1993).

4.4.3.1 Differences in feeding rates between sprat and herring

The results have only confirmed our initial hypothesis partially: at low prey concentrations of copepods (approx. < 40 L^{-1}) herring could reach higher biting rates than sprat (Figure 4-7). Unfortunately, we could not obtain a sufficient amount of data from herring preying on *A. tonsa* at higher concentrations due to the limit of the rearing facilities and the longer generation time of *A. tonsa* compared to *Artemia* nauplii. Thus, it remains uncertain whether herring also could reach higher maximum biting rate than sprat when feeding on *A. tonsa*. In contrast to experiments with copepods, the functional responses of both predators were very similar with *Artemia* nauplii.

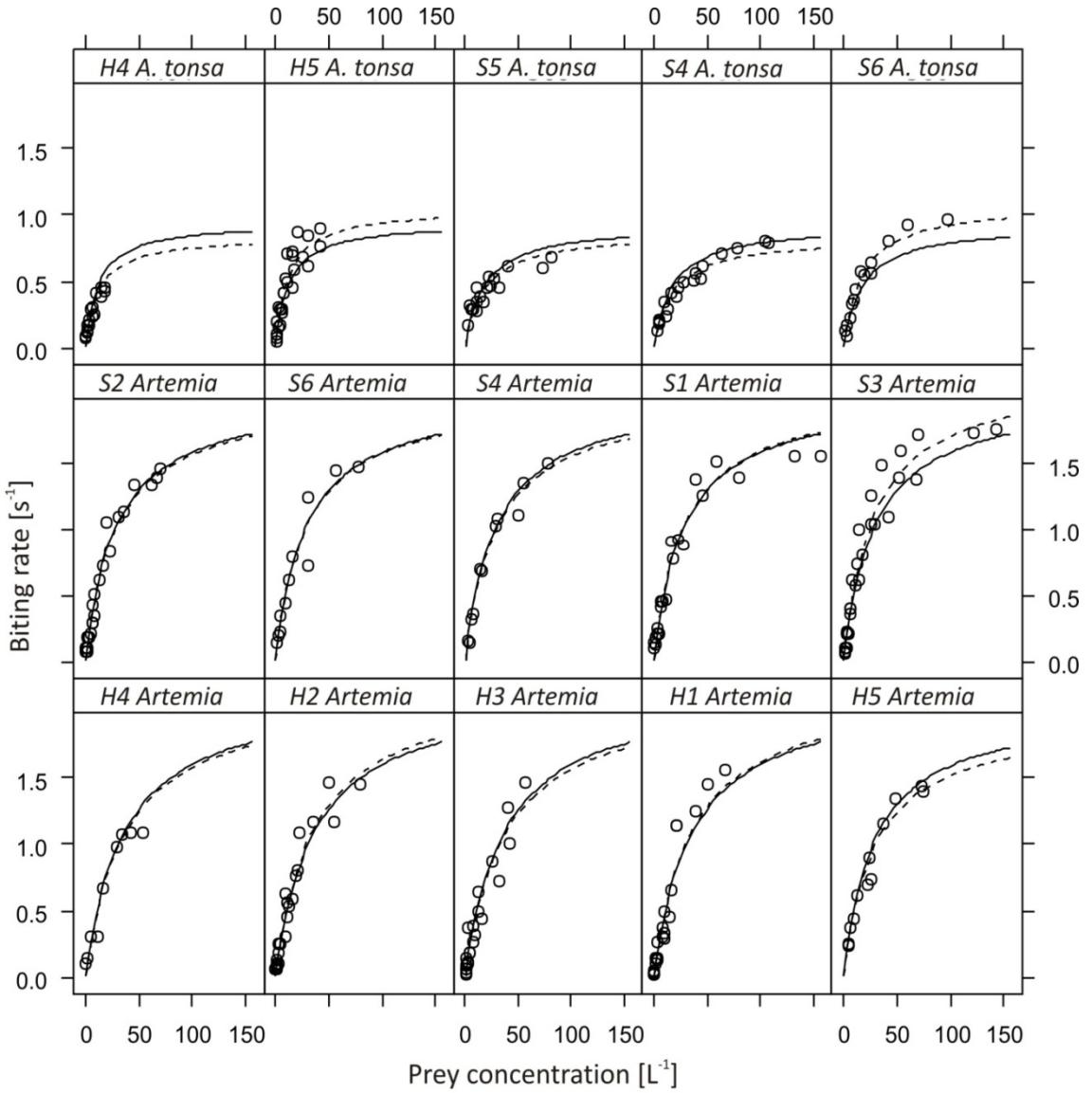

Figure 4-8 Experimental series-specific (dotted line) and population average (solid line) predicted biting rates (s^{-1}) obtained from the nonlinear mixed effects model (Table 4-3) and the corresponding observed values (circles). The plots were created automatically by the R program using the results from the mixed effects model. The scaling per each subplot could not change manually. All curves are based on the maximum concentration (x-axis) obtained. For herring this results in curves extrapolating beyond the range of measured data. Nomenclature of experimental series based on: fish species (H = herring; S = sprat) and the different fish groups (numeration)

It should be noted that herring in our experiments were slightly larger (~ 2 cm) in size than sprat. Actually, this size difference reflects the typical size structure of mixed schools of juvenile sprat and herring in the sea, where herring are on average 1-2 cm lager in size than sprat (Maes and Ollevier 2002). This size difference could have resulted in higher absolute swimming speeds of herring, allowing an increase of attack frequencies for active prey at low concentrations. Additionally, there is a growth-related change in the retina of planktivorous fish, which influences the ability to locate small particles or objects at larger distances (Blaxter and Jones 1967; Hairstone *et al.* 1982). Maes and Ollevier (2002) analysed the feeding dynamics of mixed schools of sprat and herring from the intake screens of the

nuclear power plant Doel (Schelde estuary; Belgium). They found that the feeding rate of herring was significantly higher than that of sprat (principal prey group: calanoid copepods). Furthermore, the feeding intensity of sprat decreased significantly if herring became more dominant in the mixed-species schools (Maes and Ollevier 2002).

Our functional response type II model indicates that limited food environments may favor herring over sprat. Furthermore, herring have two additional competitive advantages over sprat: with increasing body size herring start to exploit larger prey items and filter-feed. Batty *et al.* 1986 even demonstrated that herring can filter-feed in the dark, resulting in longer daily feeding times compared to sprat.

Concluding, our results suggest that juvenile herring have a competitive advantage over sprat in mixed schools when prey concentrations are low and can therefore potentially reach higher growth rates than sprat.

4.4.3.2 Prey type effects

The feeding efficiency of sprat and herring was significantly higher ($p \leq 0.0001$) with *Artemia* nauplii than with *A. tonsa* (Table 4-3, Figure 4-7). The lower feeding rates of both predators with *A. tonsa* are assumed to be mainly caused by copepod's well developed escape response compared to *Artemia* (Singarajah 1969; Trager *et al.* 1994; Kiørboe 2010). This assumption is supported by the fact that fish mostly showed an *S*-shaped curvation of the body before biting on *A. tonsa* (Figure 4-4), while this behaviour was not observed when feeding on *Artemia* (Figure 4-3 a, b). The feeding attack with *S*-shaped curvation was also described for herring (Rosenthal 1969) and anchovy (Hunter 1972) larvae. A simple straight biting attack of sprat and herring on *Artemai* nauplii took 0.12-0.16 s (Figure 4-3 a, b), which is in agreement with the result of Gibson and Ezzi (1985) for herring (mean total length 15.7 cm) feeding on *Artemia*. In contrast, one attack on *A. tonsa* with curvation of the body lasted 0.24-1.08 s (Figure 4-4). The high variability in time for feeding events with *S*-shape curvation presumably resulted from the variable spatial positions of fish in relation to the targeted prey item.

The total feeding time (duration between two biting acts) of particulate feeding planktivores includes different activities, such as search (t_s), detection (t_d), approach (t_a) and prey handling (t_h) (Figure 4-5). Searching is defined as a non-directional swimming behaviour. Detection is indirectly deduced at the transition between search and approach, while approach is a directed movement towards the prey. The term "prey handling" originated from models of predators in terrestrial systems, which spend a lot of time on capturing, processing, and digesting prey (Holling 1959, 1966). For planktivorous fish handling time is more difficult to measure since the different behavioural components are short and less conspicuous and digestion time plays no significant role. We defined handling time for *Artemia* nauplii as the time between opening and closing of the fish mouth. For *A. tonsa* handling time additionally included the time for *S*-shaped curvation of the body before biting.

Frame-by-frame analysis of sprat's feeding behaviour revealed that total feeding time decreased with increasing prey concentrations and asymptotically reached a minimum value ($t_{t\_min\ A.\ tonsa}$ ~ 1.08 s; $t_{t\_min\ Artemia}$ nauplii ~ 0.65 s) (Figure 4-5, 4-6). The curves were similar for both prey types, but the curve for *A. tonsa* was shifted to higher values at all concentrations. Paired *t*-test revealed that total feeding times at high prey concentrations (approx. > 20 L^{-1}) were significantly higher for *A. tonsa* than for *Artemia* nauplii ($p < 0.01$). Under the assumption of constant handling time per prey type, the proportion of search and approach time in total feeding time decreased with increasing prey concentrations. Hence the relative contribution of handling time increases and ultimately limits the number of prey which can be consumed in a given time (Figure 4-6). Subtracting the observed average handling time ($t_{h\ A.\ tonsa}$ = 0.48 s; $t_{h\ Artemia}$ nauplii = 0.14 s) from the average total feeding time at high prey concentrations (> 20 L^{-1}) reveal similar values for the sum of search, detection and approach times for both prey types ($t_s + t_d + t_{a\ A.\ tonsa}$ = 0.60 s; $t_s + t_d + t_{a\ Artemia}$ nauplii = 0.51 s). Hence, the observed differences between the total feeding time curves and functional response curves of both prey types were mainly caused by the different prey handling times for *A. tonsa* and *Artemia* nauplii. The estimated higher biting rates for *Artemia* nauplii are assumed to reflect the maximum possible feeding rates of non-evasive prey such as cladocerans, whereas the results of experiments with *A. tonsa* are supposed to reflect their functional response in copepod dominated environments.

The confirmation of the functional response type II for two important planktivorous species and the parameterization for two relevant prey types (evasive and non-evasive) will improve model results of end-to-end ecosystem models, like NEMURO (Megrey *et al.* 2007) or ATLANTIS (Fulton *et al.* 2004). Additionally, our results can be regarded as a first step in developing a mechanistic understanding of the interaction of plankton populations and competing planktivorous fish species.

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5 Manuscript 4: Swimming patterns of juvenile sprat and herring in relation to prey concentration and type

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Abstract

Laboratory experiments were conducted to study the effect of different prey concentrations on horizontal and vertical swimming patterns of juvenile sprat and herring. Fish were fed with various prey concentrations between 1 and 160 l^{-1} of two different single prey types. Swimming patterns and behavioural components were analysed from digital images of a top camera (for horizontal components) as well as from underwater images (for vertical components). Both fish species increased their horizontal swimming speeds, turning rates (°s^{-1}), number of sharp turns (>90°) and accelerations (cm s^{-2}) asymptotically with prey concentrations. Vertical swimming speeds (BL s^{-1}) had a great impact on the total swimming speed at concentrations >20 l^{-1}, but were less distinct at lower prey densities. All tested swimming components were lower when fish were fed with copepods instead of *Artemia*. Fish feeding on copepods showed an S-shaped body curvation during the attack, which was not performed by fish feeding on non-evasive *Artemia*. Overall had herring lower swimming speeds but higher feeding rates than sprat when both species were preying on low copepod concentrations (< 40 l^{-1}). Transferred into energy costs during foraging, the present results suggest that juvenile herring have an energetic advantage during feeding above sprat.

Keywords: sprat, herring, planktivorous fish, feeding behaviour, swimming

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5.1 Introduction

Sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) are small pelagic clupeid fish feeding on zooplankton (*e.g.* Möllmann *et al.* 2004; Hawkins *et al.* 2012) and serving as major forage fish for piscivorous predators. In the Baltic Sea both clupeids have the potential to control the biomass of lower and higher trophic levels (Möllmann and Köster 1999; Köster and Möllmann 2000; Möllmann *et al.* 2008), so that bioenergetics-based models for growth and population dynamics of these clupeids are recommended for the evaluation of their trophodynamic impact within the pelagic food web. Such models for sprat or herring still include several parameter estimates borrowed from other species (*e.g.* by Rudstam 1988; Arrhenius and Hansson 1993; 1994; Arrhenius 1998; Maes *et al.* 2005) and lack on information of swimming patterns as functions of prey densities. However, in the sense that feeding is essential for survival, a realistic model framework must include estimates of swimming patterns under different conditions.

In the past decades the zooplankton taxonomic composition in the Baltic Sea changed significantly (Möllmann *et al.* 2003; Gorokhova *et al.* 2005) which has been associated with climate-induced shifts in water temperature and salinity and increased eutrophication (Yurkovskis *et al.* 1999; HELCOM 2009). Invasive species became more abundant in recent years and are responsible for re-shaping local zooplankton communities through modified predator-prey interactions (Ojaveer *et al.* 2004; Kotta *et al.* 2006). For instance, the cladoceran *Cercopagis pengoi* became more abundant in some parts of the Baltic Sea and was found in stomachs of western Baltic sprat and herring (Gorokhova *et al.* 2004) and pelagic planktivorous originated in the Gulf of Riga (Lankov *et al.* 2010). Changes in zooplankton composition or abundance can directly influence the foraging behaviour of pelagic planktivores, which will also affect metabolic costs associated with locomotion (Helfman 1993). Juvenile Baltic herring and sprat (0-groups) inhabit mostly the shallow coastal regions of the Baltic and North Seas (Maes *et al.* 2005) and are assumed to be direct competitors utilizing the same range of prey sizes. Comparisons between sprat and herring in terms of their swimming patterns during feeding can provide important insights into the potential for competition between these two ecologically and economically important clupeids. They both prey mainly upon copepods like *Acartia tonsa*, *Pseudocalanus acuspes*, *Temora longicornis* or *Eurytemora affinis* and cladocerans like *Evadne nordmanni*, *Podon spp.* and *Bosmina spp*. (Flinkmann *et al.* 1998; Möllmann *et al.* 2004; 2005; Bernreuther 2007; Lankov *et al.* 2010). These two prey groups, copepods and cladocerans, show different escape responses to predator presence whereby calanoid copepods have a higher escape ability than cladocerans (Viitasalo *et al.* 2001) which move rather slowly. We therefore conducted laboratory experiments with sprat and herring feeding on different concentrations of two types of zooplankton prey (*Acartia tonsa* adults and *Artemia salina* nauplii) with different escape behaviours. *Artemia* nauplii are non-evasive organisms showing almost no escape response whereas *A. tonsa* adults and late copepodites react with

fast jumps and are highly sensitive to currents and turbulences induced by a predator (Robinson *et al.* 2007).

Previous laboratory studies on feeding related swimming patterns in pelagic planktivores were often focused on differences in horizontal swimming speeds between filter-and particulate feeding, *e.g.* in sardine (Garrido *et al.* 2007), Atlantic herring (Gibson and Ezzi 1985) or anchovy (James and Findlay 1989; James and Probyn 1989). Although other swimming patterns such as turning rates or frequent changes in direction were recognized during particulate feeding (James and Findlay 1989; Garrido *et al.* 2007), this was often not quantified over small scaled prey concentrations. For example, James and Findlay (1989) described increasing turning rates with increasing prey densities during particulate feeding of *Engraulis capensis*, but there were only a view values available for low densities and turns were recognized only for a few fish over short periods of time. For sprat and juvenile herring detailed information on swimming speeds or turning rates in relation to small scaled prey concentrations are not available so far. Furthermore, fast swimming speeds in the vertical plane are important behavioural components of particulate feeding in both sprat and herring (Brachvogel *et al.* 2012), but such vertical swimming speeds have not been quantified in clupeids before. Particulate feeding in sprat and juvenile herring follows a type II functional response (Holling 1959; 1966) characterized by asymptotically decelerating intake rates with increasing prey concentrations (Brachvogel *et al.* 2012). Juvenile herring can achieve higher biting rates than sprat when both species have a similar size and prey on low concentrations (< 40 l^{-1}) of copepods. Furthermore, both fish species have significantly lower feeding rates when preying on highly evasive copepods compared to feeding on *Artemia* nauplii (Brachvogel *et al.* 2012). The present study extents the results on functional response of sprat and herring presented in Brachvogel *et al.* (2012) by measurements of different feeding related swimming patterns such as mean swimming speeds (horizontal and vertical), mean turning rates, number of turns with high angles (>90°) and accelerations. These results will contribute to the development of a bioenergetics model for sprat and herring where feeding rates can be linked to foraging related swimming. Together with previous measurements of spontaneous swimming costs in sprat (Manuscript 2), the present results will allow a first approximation of energy costs associated with feeding under different conditions.

5.2 Material and Methods

5.2.1 Capture and Maintenance of experimental fish

Young-of-the-year (YoY) herring and sprat were caught by the use of a hand-operated 4m^2 dip net with a mesh size of 6 mm. Groups of fish were transported within a 700 l box with aerated seawater to the aquarium facilities at the University of Hamburg, Institute of Hydrobiology and Fisheries Science. Juvenile herring were caught in June 2009 in the Harbour of List, Sylt (North Sea; 55°01’ N 8°36’ E) at a salinity of 32 PSU and a temperature

of 14°C. Fish were maintained at the Wattenmeerstation (AWI) List in 1000 l tanks with natural seawater one week prior to the transport to Hamburg. Sprat used for experiments No 1-3 (Table 5-1) were caught in July 2009 in Kiel Bay (Baltic Sea; 54° 41' N 10° 3'E) at a salinity of 15 PSU and a temperature of 20°C. Sprat used in experiments No 4 – 6 (Table 5-1) were caught in July 2010 in Gelting (Baltic Sea; 54°75' N 9°90' E) at a salinity of 13 PSU and a temperature of 20°C. In the aquarium facilities in Hamburg all fish were maintained in artificial seawater (Aqua Medic) in circular tanks (diameter 1.5 m) in large groups of 200-500 fish under a 13 L:11 D light regime at least two weeks before starting with experiments. Two separate recirculating systems were available with mechanical and biological filter units, keeping salinity constant at 16 PSU for sprat and at 32 PSU for herring, respectively. Fish were fed daily rations of an artificial pellet diet (*Marico advance 0.5-0.8 mm*, Coppens International bv) and live freshly hatched *Artemia salina* nauplii (*SEPArt-Cysts*, INVE Aquaculture). Temperature in the seawater circulation system was kept at 16°C and regulated with a precision of ± 0.1°C.

5.2.2 Prey size and type

Two different prey organisms of similar sizes were offered in experiments:

1) *Artemia salina* nauplii were used as slow moving, non-evasive prey species (Robinson *et al.* 2007). *A. salina* cysts (INVE) were incubated in 33 PSU artificial seawater (Aqua Medic) at a temperature of 26°C for 48 h. A special separator (INVE Aquaculture) allowed the isolation of hatched animals without cysts. During experiments nauplii had a total length of ~ 830 µm.
2) *Acartia tonsa* c5- copepodites and adults (c6) were used as natural prey organisms with a high escape response (Viitasalo *et al.* 2001; Robinson *et al.* 2007). Eggs from the rearing facilities of the Institute were hatched and cultured within 240 l tanks at a salinity of 18 PSU and a temperature of 20-22°C following the descriptions in Holste and Peck (2006) and Peck and Holste (2006) at a 12 L: 12 D light regime. They were fed with *Rhodomonas* spp. twice a day and additionally shortly before the use in experiments, so that the animals had a similar colour and contrast as *A. salina*. Adults and late copepodites (c5) were collected by filtering the water from the rearing tank through a 227 µm gaze. Animals were then carefully transferred into a 5-l jar shortly before the use in experiments. During experiments copepods had a prosoma length of ~ 800 µm.

Prosoma length (*Acartia tonsa*) or total length (*Artemia salina* nauplii) of prey organisms was measured for each experiment with the measuring software ImageProPlus® (6.2) on digital images captured with a camera (Leica-300®) mounted on a binocular microscope (Leica®) at a magnification of 50.

5.2.3 Technical design of the experimental aquarium

The present study is based on the same experiments as presented in Manuscript 3 of this thesis (Brachovgel *et al.* 2012). The experimental aquarium (401 l) was placed in a separated room of the aquarium laboratories to prevent external disturbance of fish during experimentation. Temperature was constant at 16°C, salinity was kept at 16 PSU for sprat and at 30 PSU for herring experiments, respectively. The tank was divided into two sections, a smaller prey-mixing chamber (162 l) and a larger fish chamber (239 l) partitioned by a PVC-panel with two circular ports allowing water exchange between the two chambers (see also Brachvogel *et al.* 2012). The side walls of the fish tank were covered with black panels to minimize stress for the fish. The water drift was powered by the aeration behind the lower circular port in the pvc panel and the introduction of double filtered (20 and 1 µm pore diameter) artificial seawater from the recirculation system into the prey-mixing chamber. All devices needed for food introduction and export of uneaten particles were placed outside the experimental room. An electric bulb (25 W) above the tank provided a constant illumination level of 1.3 lx at the surface and 1.0 lx at the bottom of the fish tank which was sufficient to allow for particulate feeding (Batty *et al.* 1990).

5.2.4 Experimental set-up

Experiments with sprat and herring were conducted with 11 different fish groups, each consisting of 10 individuals. Overall 49 experiments with variable prey concentrations were conducted, whereby each fish group was used in 2-5 consecutive experiments (exp. series, Table 5-1) before being replaced by another group. Prey was offered in target concentrations of approx. 5, 25, 50, 100 and 200 per litre (l) in experiments with *Artemia* (Table 5-1). For experiments with copepods it was not possible to conduct multiple experiments with concentrations > 25 l^{-1} because of the limited rearing facilities and the lower reproduction rates of copepods compared to *Artemia*. Thus, only two experiments were conducted with copepod concentrations ~100 l^{-1} (Table 5-1).

Each fish group (*n*=10) was introduced into the experimental aquarium two days prior to the start of experiments. Fish were fed three hours before the experimental start with *Artemia salina* nauplii in order to test normal feeding behaviour, but allow for complete clearing of the water. Each chamber of the experimental tank (mixing chamber and fish chamber) contained a tube for food addition to achieve the specific initial target prey concentration in the whole tank. This concentration was maintained constant for 60 min by continuously supplying (peristaltic pump; 36 ml min^{-1}) a prey suspension of a known concentration into the mixing chamber. The necessary concentration of the prey suspension was calculated from the theoretical losses of food due to feeding by fish and the water flow-through (3.83 l min^{-1}). The assessment of prey loss due to feeding was initially based on biting rates observed in a pilot study. After 60 min the introduction of new prey was stopped which resulted in exponentially decreasing prey concentrations with time. Each experiment

was ongoing until either fish were not feeding any more or the prey concentration in the experimental tank was ~ 2 l^{-1}. After each experiment fish were kept in the experimental tank and water was filtered from all prey particles for at least 60 min. After this cleaning interval the next experiment started when the target prey concentration was 5 or 10 l^{-1} in the first trial. At higher concentrations only one experiment was conducted per day in order to avoid any satiation effects of the fish. To determine the actual prey concentration for each 10-min interval, all prey items lost through the overflow (3.83 l min^{-1}) were collected in a 100µm mesh-bottom cup. From this probe all prey items were counted under a binocular for concentrations <25 l^{-1}. For all other probes the number of prey was back calculated from dry weights determined for each experiment. The prey concentration (C_t) for each 10 min time interval was calculated from $C_t = N_t/FR$, where N_t is the total number of prey items (N) in the mesh-cup divided by the length of the time interval and FR is the overflow rate.

After the last experimental trial of a fish group (exp. series; Table 5-1), all animals were removed from the aquarium and rapidly killed by an overdose of anaesthetic (MS-222 at ~200 mg l^{-1}) in order to measure body mass (g) and length (mm).

Table 5-1 Overview of conducted experiments for the analysis of swimming patterns of sprat (*S. sprattus*) and herring (*C. harengus*) feeding on different single prey types (*A. tonsa* or *Artemia*) over a range of concentrations (l^{-1}). Each fish group consisted of 10 animals and was used for 3-5 consecutive experiments.

			Analysed experiments		Fish data		Prey types	
Exp No	Fish-species	exp. series	horizontal activity	vertical activity	mean WW [g]	mean SL [mm]	*A. tonsa* conc [l^{-1}]	*Artemia* conc [l^{-1}]
1	Sprat	S1	3	1	1.42 ± 0.21	54.9 ± 3.3		26;59;159
2		S2	5	2	1.45 ± 0.51	55.9 ± 2.9		8;14;32;66;70
3		S3	5	1	1.73 ± 0.22	58.0 ± 2.4		6;17;42;68;144
4		S4	4	2	1.03 ± 0.17	48.5 ± 2.7	44;104	50;78
5		S5	4	4	1.22 ± 0.47	51.0 ± 3.5	26;73	27;73
6		S6	4	3	1.87 ± 0.19	58.4 ± 3.6	26;97	30;78
7	Herring	H1	5	2	2.31 ± 0.53	66.5 ± 4.2		10;16;16;40;67
8		H2	5	2	2.49 ± 0.70	66.5 ± 4.6		16;19;21;55;79
9		H3	5	1	2.61 ± 0.48	66.3 ± 3.5	8;15;18	55;43
10		H4	5	3	3.64 ± 0.54	74.9 ± 2.5		8;16;32;41;56
11		H5	4	0	4.23 ± 0.91	76.9 ± 2.5	16;26;31;41	

Exp No = experiment number; WW = wet weight; SL = standard length, conc = maximum prey concentration at 50-60 min interval

5.2.5 Image recording

Fish were filmed from two sides with CCD-Cameras (TV7143; ABUS) under constant IR-light conditions. Thus, fish appeared as dark objects behind a light background (Figure 5-1). One camera was installed underwater and connected to a DVD recorder in a separate room. These images were captured with 25 frames s^{-1} and were used for the detection of feeding rates and the analysis of vertical swimming patterns. The second camera was installed 1.3 m above the fish tank and was connected to a PC in separate room, where images were recorded with a rate of 4 frames s^{-1} (i-Corder®; V2T Vision to Technology GmbH, Germany) and stored on hard disk. These images were used to determine horizontal movements such as mean swimming speeds, turning rates, accelerations and decelerations by the use of an automated image analysis program (fish-tracker; Manuscript 2).

5.2.6 Detection of swimming activities and feeding rates

Biting events (s^{-1}) were counted on the videos taken by the underwater-camera (Figure 5-1 C). Videos were played with half speed and a randomly chosen fish was tracked for 10-60 s while biting rates were counted. For each experimental interval 10-15 fish were tracked in this manner. Results are presented in detail in Brachvogel *et al.* (2012). The same images were used for the detection of vertical swimming speeds in body length (BL) s^{-1}. Therefore videos (25 frames s^{-1}) of some selected experiments (representative for the tested range of prey concentrations; see Table 5-1) were converted into single images. The x-y-position of a fish was obtained by marking the tip of the snout of an individual on the digital images using the program ImageJ (version 1.44p.). The fish was marked on every second frame for a complete feeding sequence which was characterized by upwards swimming while biting followed by a fast downward burst when the fish reached the surface (Brachvogel *et al.* 2012). We tracked only fish which were swimming continuously in the same distance to the camera over the individual tracking path. The fish length of each tracked fish was used as a reference calibration for the determination of swimming speed for this particular fish. We also recognized whether the fish turned its body at the surface before starting the downward burst. For each 10-min-interval 3-5 fish were tracked in this way with simultaneous counting of biting rates. We further used the tracked fish positions from the underwater images in order to estimate for the overall swimming speed. We therefore calculated an average speed for each experimental interval from 60 min onwards based on the Euclidean distance calculated for each tracked individual and the respective time period. Furthermore, we calculated a factor relating vertical to horizontal swimming speeds. Therefore distances covered in x-direction were used to determine swimming in the horizontal plane, whereas distances covered in y-direction represented vertical movements. These distances were then divided by the respective time in order to calculate swimming speeds (BL s^{-1}). Vertical swimming, however, was mostly not detectable at prey concentrations below ~20 l^{-1} and the image section of the underwater camera did not allow

quantifying horizontal swimming patterns over the lower prey concentrations adequately. Thus, images from a top-camera were used to determine horizontal swimming speeds (Figure 5- 1) allowing to relate foraging related swimming patterns to the complete range of prey concentrations provided. The investigated horizontal swimming patterns included mean swimming speeds and turning rates (°s^{-1}; number of turns>90°) and were determined by the use of an automated image analysing program running with Matlab® (The Mathworks; version 7.6.0). This program (hereafter: fish-tracker) detected the positions of fish and tracked them over the consecutive frames. All position data were stored in a database (MySQL) for further analysis. The processing of the fish-tracker is described in detail in Manuscript 2 of this thesis. The fish-tracker was modified for the new tank dimension, fish size and the fact that fish could leave the observable area of the fish tank (see Figure 5-1 A). A water velocity-model was developed for the experimental tank by measuring water velocities with an acoustic Doppler velocimeter (Vectrino, Nortek AS). This device measured 3D-water-velocities with a frequency of 25 Hz in a distance of 5 cm below the probe. The velocity model revealed that the exchange of water with prey between the mixing area and the fish tank was secured. However, this model also showed that the average horizontal water velocity over the complete water column was very low (max 0.05 cm s^{-1}). Water velocities were therefore ignored for the determination of horizontal swimming activities of the fish.

5.2.7 Calculations

For all analysis only data of swimming patterns from the 50-60-min experimental interval onwards were used (Figure 5-2). The first 60 min were used to establish a constant prey concentration and to allow fish to adapt to feeding conditions (Brachvogel *et al.* 2012). The row data (x-y-positions of fish) from the fish-tracker were analysed with a Matlab© (The Mathworks; version 7.6.0) -based routine, resulting in a csv-file with all relevant data, like the covered distance between two images or the swimming direction of the fish (see also Manuscript 2). Mean swimming speeds, turning rates (°s^{-1}) and the number of turns>90° (fish^{-1} s^{-1}) were determined for each experimental interval (á 10 min) as average value for the fish group. Mean horizontal accelerations and decelerations (cm s^{-2}) were calculated for each fish from the swimming speeds between three consecutive images:

$$\vec{a} = \Delta\vec{v}/\Delta t \qquad (1)$$

with $\vec{a}$ = mean acceleration (cm s^{-2}); $\vec{v}$ = swimming speed in cm s^{-1}; t = time in s; $\Delta\vec{v} = v(t_2) - v(t_1)$ and $\Delta t = t_2 - t_1$. Decelerations were defined as negative values. All individual accelerations for one interval were tested for normal distribution (histogram; Kolmogorov-Smirnov-test). The variance in accelerations (cm s^{-2}) per each 10 min interval was used for the analysis of a relationship between accelerations and prey concentrations.

Turning movements >90° are known to be energetically cost expensive in pelagic fishes (Enders and Herrmann 2003), correlate with oxygen consumption rates of sprat (Manuscript 2) and were recognized during feeding strikes in pike associated with s-shaped bending of the body (Harper and Blake 1991). Such turns with high angles and s-shaped body deformations were likewise observed in the present study on sprat and herring (Figure 5-1). We therefore tested the variability of turns>90° and the mean turning rate (°s^{-1}) among the offered prey concentrations.

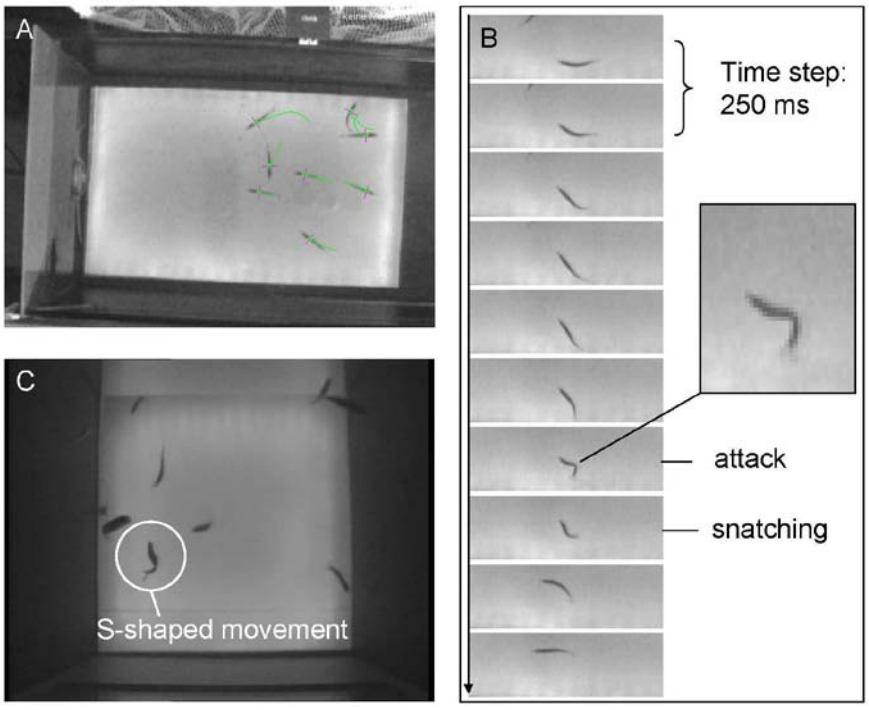

Figure 5-1 Images from the top-camera (**A**, **B**) and the underwater camera (**C**) showing sprat during feeding on copepods (*A. tonsa*). The fish-tracker program detected the position of each fish (**A**); green lines represent the positions of tracked fish on the last twelve images (**A**). The typical S-shaped curvation of the body during feeding on copepods was performed directly before snatching the prey item (**B**, **C**)

5.2.8 Model fitting for horizontal swimming patterns

We modelled horizontal swimming speeds, accelerations and turning rates for herring and sprat as a function of prey concentration for the two prey types offered. We therefore used a *mixed effects* model (Pinheiro and Bates 2000), which incorporates both *fixed effects,* which are parameters associated with an entire population (here: fish species or the prey type) and *random effects*, which are associated with individual experimental units drawn at random from a population (here: experimental series). The approach was to utilize a nonlinear model of the form

$$y = f(x_{ij}, \beta, u_i) + e_{ij} \quad (2)$$

where f is a nonlinear function of the prey concentration (x_{ij}) for the jth observation (response variable) on the ith subject (exp. series), unknown fixed effect parameters (β), an unknown vector of random effect parameters (u_i), and unknown random errors (e_{ij})

(Davidian and Giltinan 1995, Peek *et al.* 2002). For our experiments, the fixed effect (β) treatment design is a 2 x 2 factorial with two fish species (*S. sprattus* and *C. harengus*) and two prey types (*A. salina* and *A. tonsa*) with an unbalanced design as we had not exactly the same prey concentrations among the different experiments.

Taking repeated measurements on the same fish groups (exp. series) at different prey concentrations necessitates incorporation of random effects (u_i). If random effects were not included in the model, the assumption of independent error terms would be violated, producing biased standard errors. The nonlinear function (*f*) we used in equation (2) was a Gompertz function (Gompertz 1825) of the form:

$$y(x) = \phi_1 + (\phi_2 - \phi_1) \exp [- \exp(\phi_3)x] \quad (3)$$

Thereby *y* represents the response variable, which was either the mean horizontal swimming speed (cm s^{-1}; BL s^{-1}), the number of turns>90° ($fish^{-1}s^{-1}$), the mean turning rate ($°s^{-1}$) or the variance in accelerations (cm s^{-2}). The symbol *x* denotes the prey concentration (explanatory variable), ϕ_1 is a parameter representing the asymptotic maximum and ϕ_2 the parameter representing the intercept where y =0. The parameter ϕ_3 is the logarithm of the rate constant representing the ascending slope until the asymptote is reached. The corresponding half-life $t_{0.5}$ = log2/exp(ϕ_3).

We used a nonlinear mixed effect model procedure in R (version 2.13; R development core team 2011) with the nlme-package (version 3.1-101; Pinheiro *et al.* 2011) to fit curves to swimming activity data from the different fish groups using equation (3) with the fixed and random effects in equation (2). The modelling procedure followed the descriptions and recommendations of Pinheiro and Bates (2000) and Zuur *et al.* (2009a). We used a self-starting function (SSasymp) available from the nlme package in R, which allows to start the modelling process without providing initial values for ϕ_1, ϕ_2 and ϕ_3 (Eq. 3). As covariates we used fish species (*S. sprattus* and *C. harengus*) and prey types (*A. salina* and *A. tonsa*). For each of these covariates different fixed effect parameter estimates can be determined in the modelling process, allowing to evaluate differences in swimming patterns (response variables) in relation to prey concentrations (explanatory variable) between fish species or prey types, respectively.

In a first step we used a model with the same fixed effect parameter estimates for both prey types and fish species, respectively, but with random effects included for all three parameters. When random effects were correlated with each other this indicated that the model was over-parameterized (see Pinheiro and Bates 2000). Alternative models with only one or two random effects each were compared with AIC criteria and highest significance (*p*-values). In a next step, we tested the inclusion of different variance-covariance structures in order to account for heterogeneity of variance within group errors using covariates. We chose *varIdent* as the best fit (lowest AIC) structure (Pinheiro and Bates 2000) for the swimming speed model and turning rate model I ($°s^{-1}$; Table 5-2). The *varIdent* model specified a variance function for the random effects with different variances for each level of

prey type and fish species, respectively. For the acceleration model (Acc Model; Table 5-2) and the Turn Model II (turns>90°) a *varIdent* model was included, which only considered different variances for the prey type levels.

After random effects were included, the next step was to introduce the covariates in the fixed effects part. This was done in a step-wise manner by first allowing only the parameter $ø_1$ to vary among prey types and fish species and then the other parameters, respectively. Different models with one, two or three of the parameters included in the fixed part to vary with species and prey type were compared via ANOVA. The best-fit model (in terms of lowest AIC and BIC and parameter significance) was than chosen as final model. Afterwards, we compared the parameter estimates obtained from the final model with a generalized least squares model (gnls in R) by a fixed effect ANOVA in order to test for the necessity of incorporation of random effects (Pinheiro and Bates 2000, Peek *et al.* 2002). Homogeneity of residuals of the final nlme-model was tested by a Shapiro-Wilk test and plots of residuals versus fitted values.

Extreme outliers were excluded for the modelling process when these values caused the algorithms to fail and the enhancement of the number of iteration steps was not sufficient to solve this problem. The handling and definition of outliers followed recommendations by Zuur *et al.* (2009b) and was based on graphical tools.

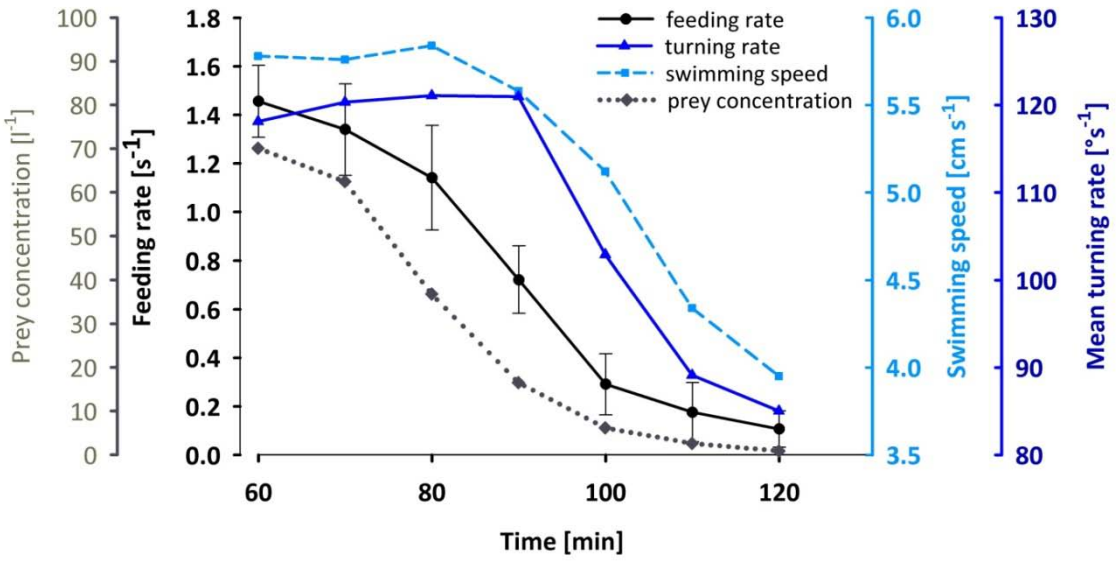

Figure 5-2 Example of mean horizontal swimming speeds (cm s^{-1}) and turning rates (°s^{-1}) of sprat during feeding on *Artemia salina* nauplii and the respective feeding rates (error bars indicate standard deviations) and prey concentrations over experimental time (experimental series S6; Table 5-1). The first 60 min of experiments allowed establishing a constant prey concentration and fish adapting to feeding conditions in the tank and were not used for later analyses

5.2.9 Transferring foraging related swimming patterns into energy costs

In order to make conclusions about the total energy costs during feeding, we combined the present results of feeding related swimming patterns with a model for energy costs associated with spontaneous swimming in sprat (M_{O2}; Manuscript 2, here as Eq. 4):

$$M_{O2}\ (mg\ O_2\ fish^{-1}h^{-1}) = 0.032\ WW^{1.073}\ e^{(0.078\ T)} + 0.533\ U^{3.281}\ e^{(0.139\ T)} + 0.859\ M \quad (4)$$

with WW = wet weight (g), T = temperature (°C), U = swimming speed (BL s^{-1}) and M = number of turns>90° ($fish^{-1}s^{-1}$). We first determined the mean horizontal swimming speeds (BL s^{-1}) and numbers of turns>90° from the nonlinear mixed effects models (Eq. 2 and 3) for each concentration and for each fish species and prey type, respectively. Afterwards, these values were implemented as U and M into equation (4) for each prey concentration >20 l^{-1}. To approximate for the energy costs of feeding at high prey concentrations (> 40 l^{-1}) we needed to add the costs for the vertical swimming speeds. However, the direct application of equation (4) resulted in unreasonable high MO_2 values when extrapolated beyond the measured range of swimming speed values in the respirometry study (0.28-0.74 BL s^{-1}). This was basically an effect of the high swimming speed exponent of *v* = 3.281 and the interaction of swimming costs with temperature (Eq. 4). We therefore used a slightly modified metabolic rate model where turns were not considered as they are already included the horizontal component (Eq. 4). Furthermore, a new swimming speed exponent was estimated (nls; R development Core Team 2011) on the basis of respiration rate values taken at 16°C (Manuscript 2) similar to the present experiments. This procedure allowed modelling costs for higher swimming speeds as measured during the respirometry study (Manuscript 2). The resulting model for the costs of vertical swimming included the *standard metabolic term* adopted from Manuscript 2 and a new estimation of the energy **costs for vertical swimming** with higher speeds:

$$MO_2\ (mg\ O_2\ fish^{-1}h^{-1}) = \mathit{0.032\ WW^{1.073}\ e^{(0.078\ T)}} + \mathbf{3.04\ U^{1.55}} \quad (5)$$

Metabolic rates were transferred into energy equivalents (Joules) by the use of an oxycaloric equivalent of 13.72 $mgO_2\ J^{-1}$ (Elliott and Davison 1975). The energy intake at a given prey concentration was calculated by the use of the functional response curves presented in Brachvogel *et al.* (2012) for both clupeids and the two prey types, respectively. For feeding on copepods the energy content of one prey item was adopted from *A. clausii* adults as 0.169 J $individual^{-1}$ (Kerambrun 1987) and for fish feeding on non-evasive prey we used the energy content of a large cladoceran (0.62 mm length), *Bosmina coregoni*, of 0.102 J $individual^{-1}$ (Vijverberg and Frank 1976) to calculate the total energy intake during feeding (J $fish^{-1}h^{-1}$).

5.3 Results

5.3.1 Feeding behaviour

Before food was introduced, both herring and sprat were swimming with low swimming speeds (0.31-0.66 BL s^{-1}) in a loose school. After food introduction the school broke, swimming speeds increased by a factor of up to 2.1 at the highest prey concentrations and all fish fed individually. Both, herring and sprat showed particulate feeding during all conducted experiments. Thereby, fish visually selected prey items in short distance to the eyes and ingested prey particles located in the upper region of their visual field. This behaviour was visible on the tracked positions from the underwater camera images (Figure 5-1). When sprat or herring were feeding on copepods, they showed an S-shaped curvation of their body directly before the attack (Figure 5-1), which was not observed in experiments with *Artemia* as prey. For sprat no filter-feeding or intermediate feeding behaviours were observed, but herring were occasionally filter-feeding or gulping at *Artemia* nauplii concentrations >50 l^{-1}. These filter-feeding events were observed much less frequently than biting rates and hard to detect quantitatively on the underwater images.

5.3.2 Horizontal swimming speeds

Both herring and sprat increased their horizontal swimming speeds (BL s^{-1}) with increasing prey concentrations (l^{-1}) until a concentration of 30-39 prey particles l^{-1} (Figure 5-3). Beyond this concentration mean swimming speeds reached a plateau for both sprat and herring, regardless of the prey type (Figure 5-3). The nonlinear mixed effects model for explaining mean horizontal swimming speeds (SS Model I) as a function of prey concentration and prey type revealed a significant difference in the asymptotic maximum swimming speed ($ø_1$) between herring and sprat when both were feeding on *Artemia* and within each fish species between the two prey types offered. Herring feeding on *A. tonsa* had lower maximum swimming speeds of 0.81 BL s^{-1} compared to herring feeding on *Artemia*, with a speed of 0.96 BL s^{-1}. A similar relationship was observed for sprat with a maximum speed of 0.92 BL s^{-1} when feeding on *A. tonsa*, but a higher maximum speed during feeding on *Artemia* with 1.07 BL s^{-1}. Swimming speeds in sprat reached their maximum values at a concentration >38 copepods l^{-1} and at >39 *Artemia* l^{-1}, respectively. For herring, maximum speeds were observed at copepod concentrations >32 l^{-1} and *Artemia* concentrations >30 l^{-1}, respectively. Below a concentration of ~30 l^{-1}, no significant differences were observed for mean swimming speeds between sprat and herring feeding on the same prey type, nor were there any significant differences within a species feeding on different prey organisms. Thus, the parameters $ø_2$ and $ø_3$ in equation (3) were modelled as constant values for both fish species and prey types, respectively (Table 5-2). Random effects were, however, included for the parameters $ø_3$ and $ø_2$ as the exclusion of one of these parameters from random effects resulted always in a poorer model fit (according to AIC, BIC

and logLIK). The included random effects allowed for the consideration of differences in swimming speeds at lower concentrations (<~30 L^{-1}) between different experimental series (fish groups), which could not be explained by the covariates (prey type and fish species).

5.3.3 Turning rates

We tested mean turning rates ($°s^{-1}$) and the number of turns>90° ($fish^{-1}s^{-1}$) in relation to the provided prey concentrations. Both variables increased asymptotically with prey concentrations in both fish species and for both offered prey types, respectively (Figure 5-4 and 5-5). The final mixed effects model for turning rates (Turn Model I; Table 5-2) revealed no significant differences in the asymptotic maximum values ($ø_1$) between sprat feeding on *A. tonsa* and sprat feeding on *Artemia* ($ø_{1\ ARTEMIA}$ = 125.23; $ø_{1\ COPEPOD}$ = 124.99). However, for herring this parameter estimate was significantly smaller when fish were feeding on copepods ($ø_{1\ COPEPOD}$ = 116.16) than for fish feeding on brine shrimp nauplii ($ø_{1\ ARTEMIA}$ = 134.63), although the data appear to be not very different (Figure 5-4). The asymptotic parameter $ø_1$ was also significantly higher in herring than in sprat when both species were preying on *Artemia,* but significantly higher for sprat when both clupeids fed on *A. tonsa*. Besides $ø_1$, also $ø_2$ (Eq. 3) was modelled to vary between fish species and prey types, but this parameter was only significantly different between sprat and herring (Table 5-2) and not between the two prey types offered. However, the exclusion of $ø_2$ from the fixed effects part or the exclusion of the prey-type: fish-species interaction resulted in a model with a poorer fit, so that the final model includes separate estimates for both, fish species and prey types. The parameter $ø_3$ was neither different between sprat and herring nor between the two prey types, so that this parameter was modelled as constant value. Random effects were included for $ø_1$ and $ø_2$ and accounted for the variability in these parameters among the different experimental series.

The final mixed effects model for the number of turns>90° revealed no significant effects of fish species or prey types, respectively (Figure 5-5). Thus, the best-fit model included no covariates in the fixed part (Turn Model II; Table 5-2), resulting in constant estimates for all three parameters among both fish species and prey types, respectively. However, random effects were included for the asymptotic maximum values ($ø_1$) and allowed considering differences between experimental series. Some outliers occurred for herring feeding on *Artemia*, but the exclusion of these values did not influence the overall conclusion of no species and prey effects on the number of turns>90°, respectively. The asymptotic maximum number of turns>90° ($ø_1$) was 0.367 ($fish^{-1}s^{-1}$) for both prey types and fish species, respectively (Figure 5-5).

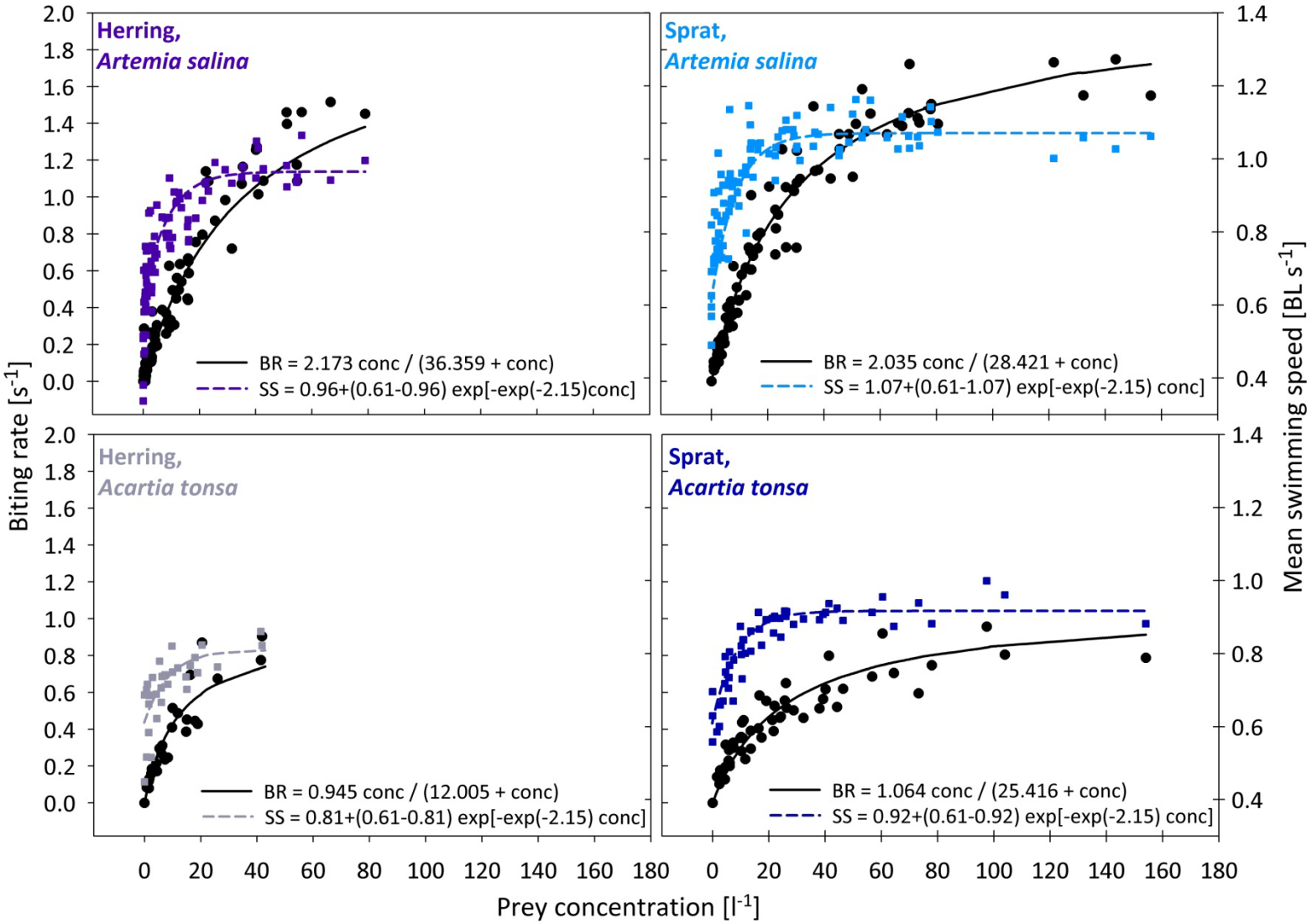

Figure 5-3 Relationships between mean swimming speeds (SS; BL s^{-1}) and biting rates (BR; s^{-1}) over different prey concentrations (l^{-1}) for sprat and herring feeding on either copepods (*Acartia tonsa*) or *Artemia salina* and the corresponding nonlinear functions. Body lengths were calculated as mean values for each fish species (sprat = 5.45 cm; herring = 7.02 cm). Black dots and lines represent biting rates and were adopted from Brachvogel *et al.* (2012). Coloured squares represent the mean swimming speed of the fish group for the same experimental interval as biting rates. Details on the parameter estimates for the swimming speed models (dotted lines) are provided in Table 5-2

Table 5-2 Summary of statistics and parameter estimates for nonlinear mixed-effects models (eq. 2 and 3) describing horizontal activity rates (response variable) of sprat and herring as a function of concentrations (conc; l^{-1}) of either copepods (*Acartia tonsa*) or brine shrimp nauplii (*Artemia salina*). As response variables (y) were used: relative swimming speeds (SS Model I; BL s^{-1}), absolute swimming speeds (SS Model II; cm s^{-1} turning rates (Turn Model I; °s^{-1}), variance in accelerations (Acc Model; cm s^{-2}) and number of turns>90° (Turn Model II; $fish^{-1}$ s^{-1}). For details on the modelling process and parameter descriptions see text. Model: y(x) = ø1 +(ø2- ø1) exp[-exp(ø3) conc]. DF = degrees of freedom; Std. Error = standard error; Std. Dev = standard deviation; overall *p*-values were determined via ANOVA of the respective model, BL = total body length

		Fixed effects						overall	**Random effects**	
Model	Response y(x)	Parameter	estimate	Std. Error	t-value	*p*-value	DF	*p*-value	parameter	Std. Dev
SS Model I	swimming	ø1 Herring : Artemia	0.965	0.017	57.77	0.000	249	<0.001		
	speed (BL s^{-1})	ø1 Herring :Copepod	0.809	0.023	-6.88	0.000		<0.001		
		ø1 Sprat : Artemia	1.070	0.018	5.73	0.000		<0.001	ø2	0.072
		ø1 Sprat : Copepod	0.917	0.026	0.08	0.935		0.7406	ø3	0.364
		ø2	0.614	0.021	29.44	0.000		<0.001		
		ø3	-2.15	0.12	-18.06	0.000		<0.001		
SS Model II	swimming	ø1 Herring : Artemia	6.76	0.11	59.51	<0.001	249	<0.001		
	speed (cm s^{-1})	ø1 Herring : Copepod	5.68	0.16	-6.92	<0.001		<0.001		
		ø1 Sprat : Artemia	5.83	0.12	-7.65	<0.001		<0.001	ø2	0.498
		ø1 Sprat : Copepod	5.00	0.17	0.17	0.1515		0.2382	ø3	0.368
		ø2	3.71	0.14	26.33	<0.001		<0.001		
		ø3	-2.14	0.12	-17.87	<0.001		<0.001		
Turn Model I	mean turning	ø1 Herring : Artemia	134.63	4.17	32.26	0.000	246	<0.001		
	rate (°s^{-1})	ø1 Herring : Copepod	116.16	6.12	-3.02	0.003		<0.001		
		ø1 Sprat : Artemia	125.23	4.88	-1.93	0.055		<0.001		
		ø1 Sprat : Copepod	124.99	7.76	2.35	0.020		0.8661	ø1	6.177
		ø2 Herring : Artemia	86.73	2.55	33.96	0.000		<0.001	ø2	4.017
		ø2 Herring : Copepod	87.97	3.99	0.31	0.756		0.0004		
		ø2 Sprat : Artemia	93.72	3.43	2.04	0.043		0.0345		
		ø2 Sprat : Copepod	94.36	5.30	1.44	0.152		0.725		
		ø3	-2.91	0.11	-25.44	0.000		<0.001		
Turn Model II	turns>90°	ø1	0.36	0.02	20.01	0	248	<0.001		
	($fish^{-1}s^{-1}$)	ø2	0.22	0.01	41.39	0		<0.001	ø1	0.055
		ø3	-3.13	0.18	-17.10	0		<0.001		
Acc model	variance of	ø1 Herring : Artemia	465.99	18.25	25.53	0.000	243	<0.001		
	acceleration	ø1 Herring : Copepod	290.24	53.74	-6.49	0.000		<0.001	ø1	35.638
	(cm s^{-2})	ø1 Sprat : Artemia	424.77	21.67	-1.90	0.058		0.064	ø2	35.392
		ø1 Sprat : Copepod	335.23	34.44	2.50	0.013		0.005	ø3	0.397
		ø2	154.17	10.77	14.31	0.000		<0.001		
		ø3	-2.55	0.13	-19.39	0.000		<0.001		

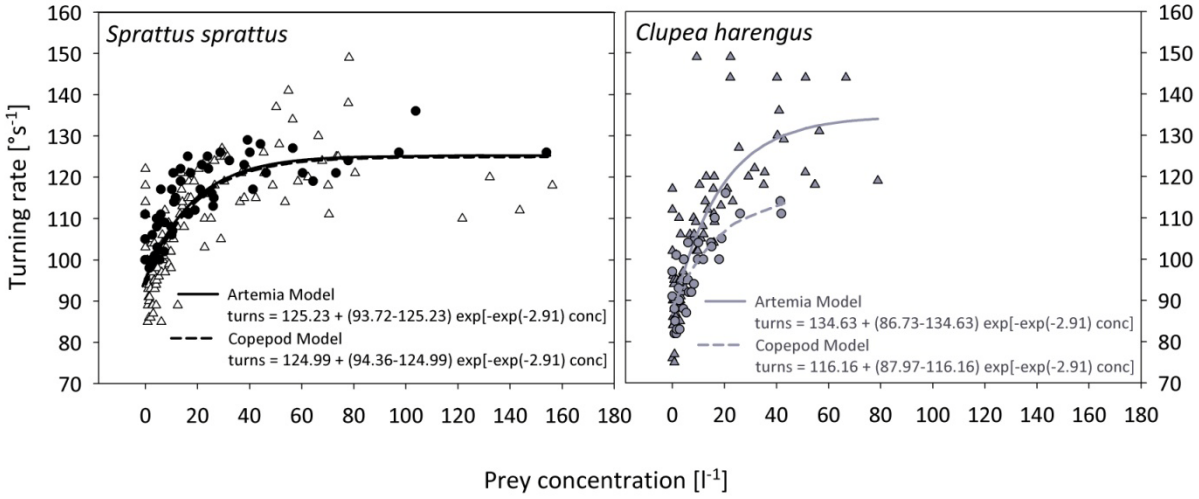

Figure 5-4 Relationships between mean turning rate ($°s^{-1}$) for sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) feeding on different concentrations (l^{-1}) of either copepods (*Acartia tonsa*; circles, dotted lines) or *Artemia salina* (triangles, solid lines) and the corresponding nonlinear functions. Statistics for the parameter estimates of the turning rate models are provided in Table 5-2 (Turn-Model I)

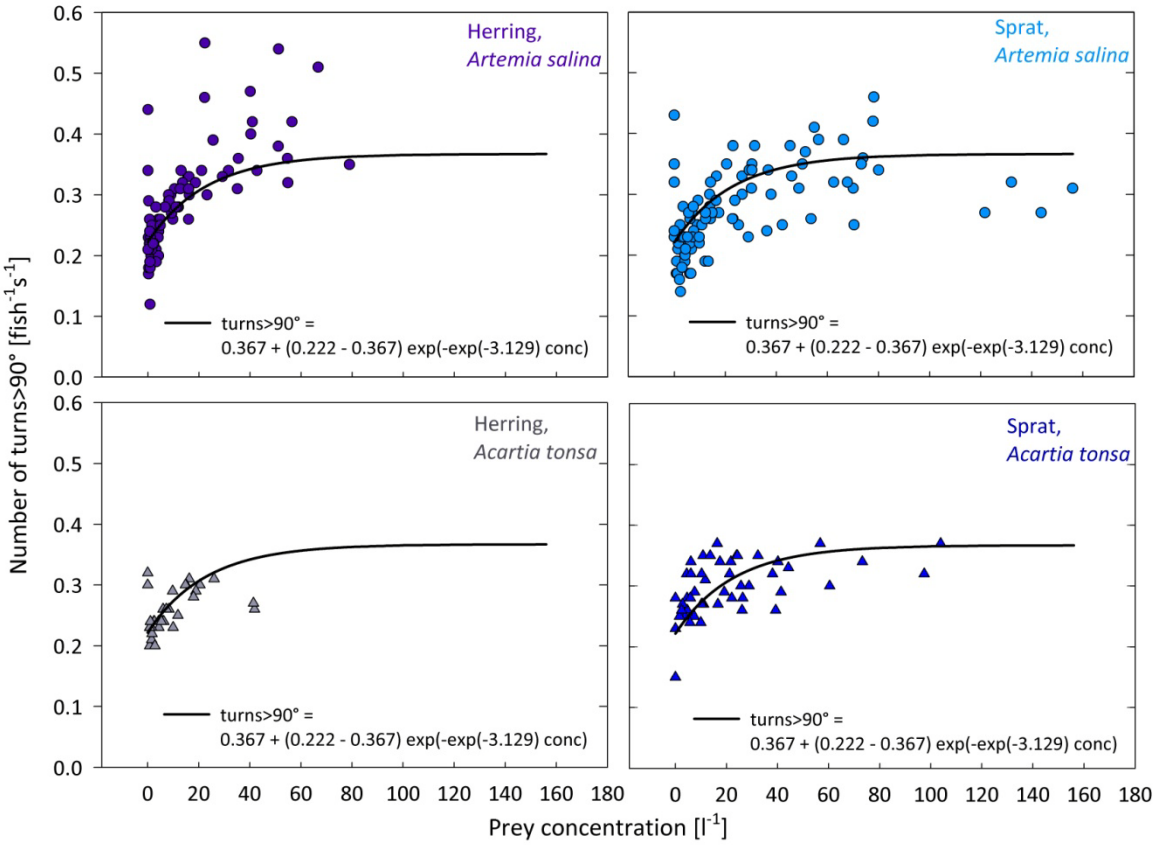

Figure 5-5 Relationships between the number of turns>90° for sprat and herring feeding on different concentrations (l^{-1}) of either copepods (*Acartia tonsa*; triangles) or *Artemia salina* (circles) and the corresponding nonlinear function (black curve) with the same parameter estimates for all covariates, meaning that there are neither significant differences between sprat and herring nor between the two prey types offered. Statistics for the parameter estimates are given in Table 5-2 (Turn Model II)

5.3.4 Horizontal acceleration

For each experimental interval, accelerations (cm s^{-2}) and decelerations were calculated per fish and visualised by frequency distribution plots. These plots revealed, that at higher concentrations (>40 l^{-1}), high values for accelerations and decelerations were found more frequently than during intervals with low concentrations (<15 l^{-1}) or without food supply (Figure 5-6). As mean values were always close to zero, we used the variance in accelerations as response variable to test against prey types and concentrations (Acc Model, Table 5-2) and fitted asymptotic curves to these data using equations (2) and (3) by a nonlinear mixed effects procedure. The best fit model (in terms of AIC, BIC and LogLIK) resulted in constant estimates for all species and prey types for the parameters $ø_2$ and $ø_3$, but separate estimates for the asymptotic maximum value $ø_1$ for each covariate. However, the difference in the $ø_1$ estimates between sprat and herringwas not significant when both species fed on Artemia ($ø_{1\ SPRAT:ARTEMIA}$ = 425; $ø_{1\ SPRAT:ARTEMIA}$ = 466), but for herring feeding on *A. tonsa* $ø_1$ was significantly lower (290) than for sprat with $ø_1$ = 355 (Table 5-2; Figure 5-7). For both species, maximum acceleration values were lower when fish fed on copepods instead of *Artemia*. Therefore, the final model included the parameter $ø_1$ in the fixed effects part for both covariates (fish species and prey type). Random effects were, however, included for all three parameters, as models with less parameters in the random part had always significantly higher values for AIC and BIC and had more outliers in residual plots.

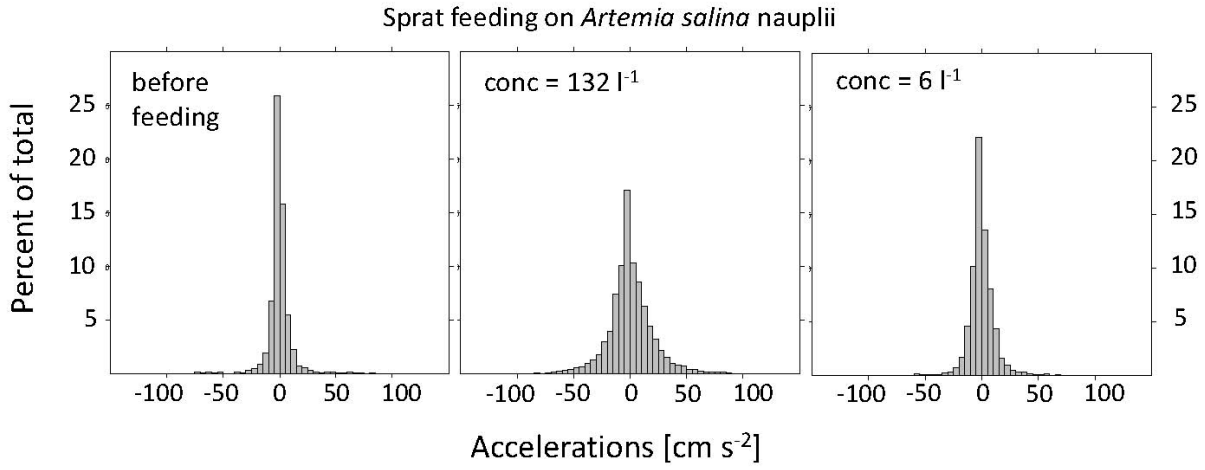

Figure 5-6 Example of the frequency distribution of horizontal accelerations (cm s^{-2}) detected for sprat from a feeding-experiment with *Artemia salina* nauplii as prey source. Left panel represents the observed accelerations before feeding, middle panel at a high and right panel at a low concentration (conc), respectively. Negative values were interpreted as decelerations

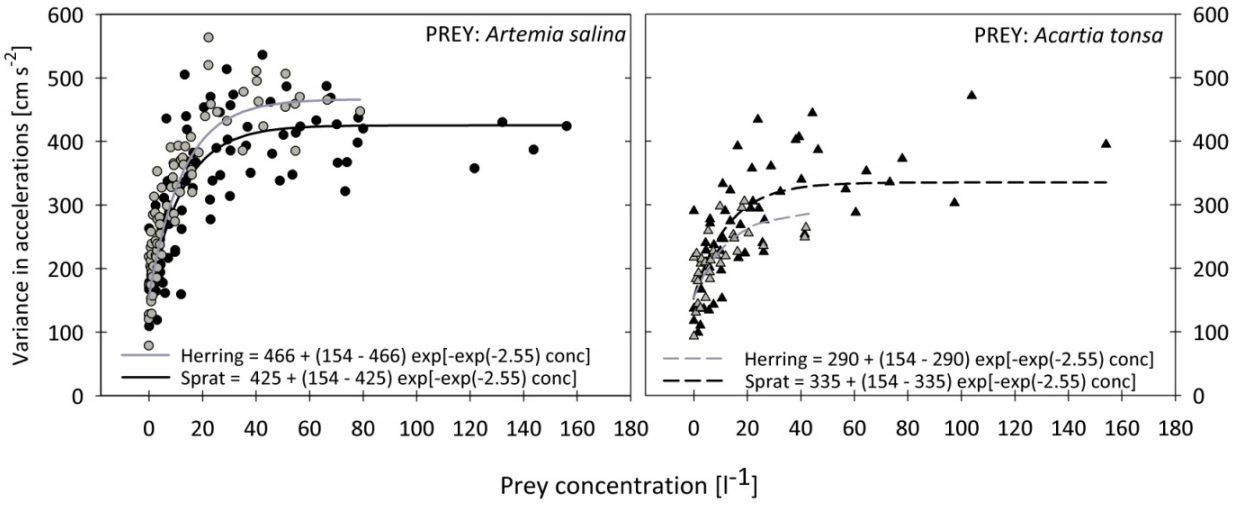

Figure 5-7 Variance in horizontal accelerations (cm s^{-2}) for sprat (black symbols) and herring (grey symbols) feeding on either *Artemia* (left panel) or *A. tonsa* (right panel) in relation to prey concentrations (l^{-1}) and the corresponding asymptotic functions. Details on parameter estimates are given in Table 5-2

5.3.5 Vertical swimming patterns

During feeding on *Artemia* nauplii at concentrations below ~ 15 l^{-1}, vertical swimming speeds represented a substantial part of the total swimming activity in sprat and herring, respectively. One individual feeding event at prey concentrations higher ~15 l^{-1} was characterized by an upward swimming during feeding, followed by a single turn at the surface and a fast downward burst before upward swimming was started again (Figure 5-8).

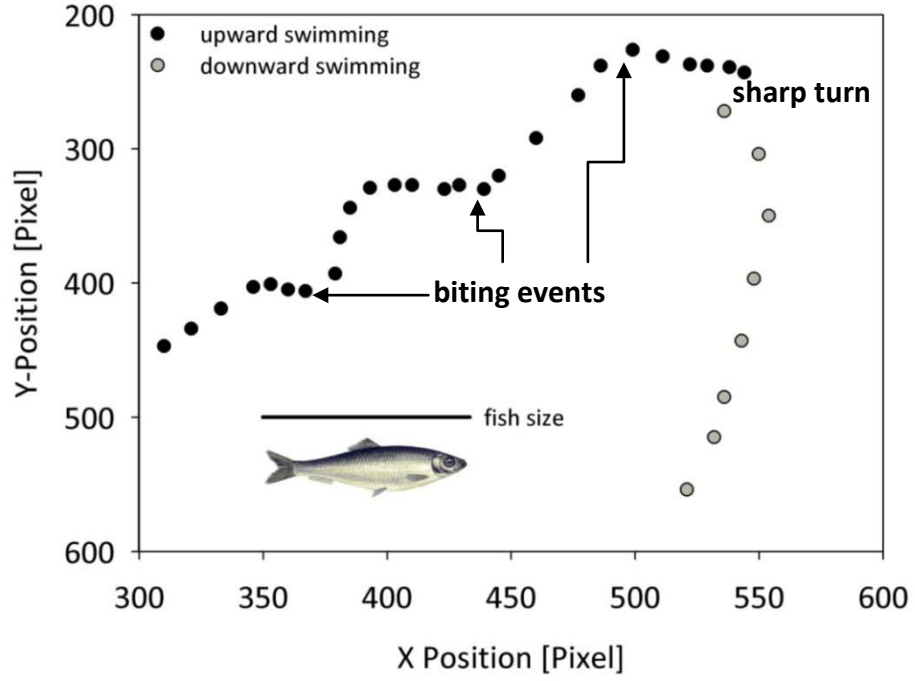

Figure 5-8 Vertical x-y positions of sprat representing one typical vertical swimming pattern during feeding on *Artemia salina* nauplii at a concentration of 28 l^{-1}. Each point was detected on images obtained by an underwater camera. The time step between each measurement was 0.08 s. The fish size (here 6.7 cm TL) was used as a reference for calculating the vertical swimming speed (BL s^{-1}) for the particular fish

At lower prey concentrations (<15 l^{-1}) fish spent more time for searching new food particles and the typical vertical swimming behaviour was performed less frequently (see also Brachvogel *et al.* 2012), so that no significant vertical movements were detected with our methods. Vertical swimming speeds were calculated from changes in Y-Positions with time, separated for upwards and downwards movements, respectively (Figure 5-8). For sprat feeding on *Artemia* vertical swimming speeds ranged from 0.42 to 2.98 BL s^{-1} during upward swimming and from 0.84 to 9.91 BL s^{-1} during downward bursts (Figure 5-9). When sprat were feeding on *A. tonsa*, vertical swimming speeds were lower and ranged from 0.62 to 1.67 BL s^{-1} during upward swimming and from 2.53 to 7.17 BL s^{-1} during downward swimming, respectively. Compared with sprat, herring showed slightly lower vertical swimming speeds when feeding on *Artemia* at concentrations between 22 and 104 l^{-1} with values ranging from 0.84 to 1.79 BL s^{-1} during upward swimming and 2.49 to 8.01 BL s^{-1} during downward swimming, respectively. For feeding on *A. tonsa*, vertical swimming speeds of herring were not detectable due to the low prey concentrations offered in experiments (mainly <20 l^{-1}).

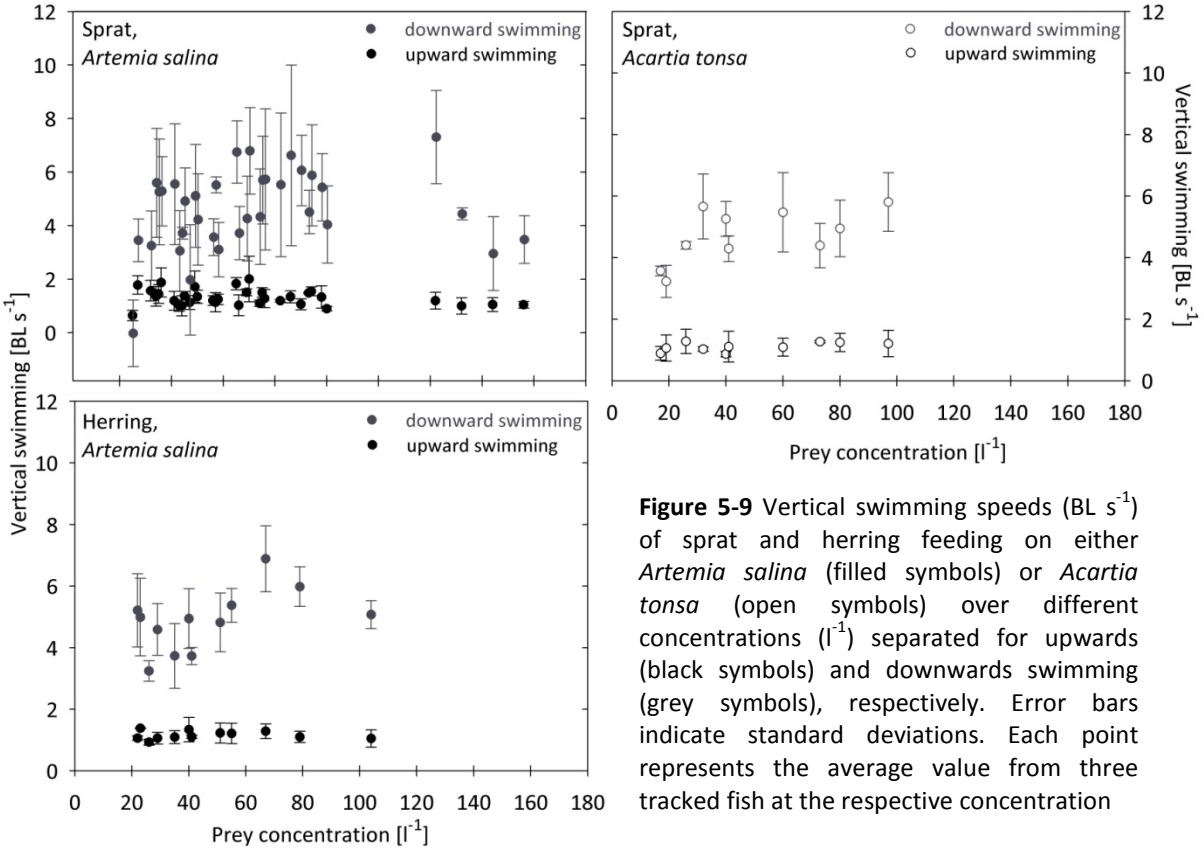

Figure 5-9 Vertical swimming speeds (BL s^{-1}) of sprat and herring feeding on either *Artemia salina* (filled symbols) or *Acartia tonsa* (open symbols) over different concentrations (l^{-1}) separated for upwards (black symbols) and downwards swimming (grey symbols), respectively. Error bars indicate standard deviations. Each point represents the average value from three tracked fish at the respective concentration

The overall mean swimming speed included speeds in the vertical and horizontal plane and was calculated from the changes in xy-positions (Euclidean distance) of vertically tracked fish as average value of upwards and downwards swimming (Table 5-3). These speeds were slightly lower for sprat feeding on *A. tonsa* (2.92 ± 0.52 BL s^{-1}) than for sprat preying on *Artemia* (3.39 ± 0.73 BL s^{-1}). In herring, the mean overall swimming speed during

feeding on *Artemia* was lower (3.10 ± 0.51 BL s^{-1}) compared to sprat. The factor relating vertical (changes in y-positions) to horizontal swimming (changes in x-positions) speeds revealed that vertical swimming speeds were on average 1.49 - 1.67 times higher than the simultaneous horizontal movements in sprat and 1.04-times higher in herring (Table 5-3).

Table 5-3 Overview of swimming speeds detected from xy-positions of tracked fish from images of the underwater camera (side view). For each fish species: prey type pair, mean swimming speeds (BL s^{-1}) and the ratio between vertical and horizontal movements (y/x speeds) is given. Mean overall speeds were calculated by the Euclidean distance obtained from the xy-positions of a fish between two images. Upwards and downwards speeds referred to changes in y-positions only

	Swimming speeds detected from underwater camera (side view)			
Fish species: prey type	Mean overall speed [BL s^{-1}]	Mean upwards speed [BL s^{-1}]	Mean downwards speed [BL s^{-1}]	Ratio between y / x speeds
Sprat: *Artemia salina*	3.39 ± 0.73	1.31 ± 0.39	5.58 ± 1.74	1.67 ± 0.44
Sprat: *Acartia tonsa*	2.92 ± 0.52	1.10 ± 0.30	4.70 ± 1.15	1.49 ± 0.54
Herring: *Artemia salina*	3.10 ± 0.51	1.15 ± 0.24	4.80 ± 1.22	1.04 ± 0.69

5.3.6 Foraging related energy costs

Foraging related energy costs were calculated by the application of metabolic rate models where horizontal (Eq. 4) and vertical swimming patterns (Eq. 5) from the present study were implemented. As horizontal swimming was dominant at lower prey concentrations and significant vertical swimming occurred only at concentrations >20 l^{-1}, two different models were applied, one for low (Eq. 4; Figure 5-10) and the other for high prey concentrations (Eq. 5; Figure 5-11), respectively. For both fishes total energy costs during feeding were lower for evasive prey at the same prey concentration (<20 l^{-1}) compared to costs for feeding on non-evasive prey (Figure 5-10 a, b). When feeding on non-evasive prey, sprat showed higher swimming speeds (BL s^{-1}) and had therefore higher foraging costs compared to herring (Figure 5-10 a, c). The metabolic costs for foraging were subtracted from the energy intake rates in order to assess the net available energy (Figure 5-10 c, d). This calculation revealed that there is no clear difference in the energetic efficiency of feeding between sprat and herring when both species are preying on non-evasive prey (Figure 5-10 c), but herring have a clear feeding advantage above sprat when both species preyed on copepods (Figure 5-10 d).

The net energy available during preying on high concentrations of either evasive or non-evasive prey was exemplary illustrated for a concentration of 70 l^{-1} (Figure 5-11). Overall 50-63% of the total energy intake during feeding was spend for swimming costs in both sprat and herring, whereby vertical swimming speeds accounted for 33% of the total energy intake of sprat feeding on non-evasive prey and for 25% when sprat preyed on copepods (Figure 5-11). In contrast, herring invested only 27% of the ingested energy into the vertical swimming speeds when preying on non-evasive prey.

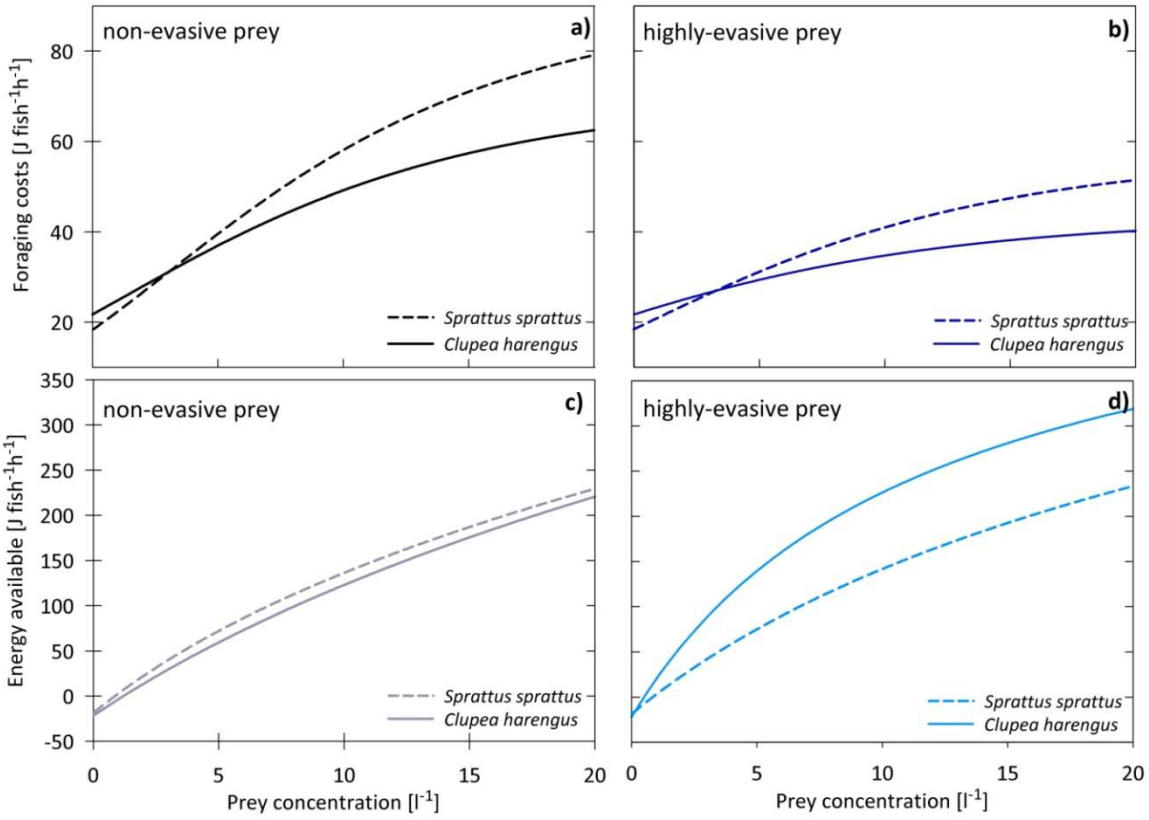

Figure 5-10 Energy costs (J fish^{-1}h^{-1}) of sprat and herring for foraging related swimming (**a**, **b**) and the netto available energy calculated by energy intake minus foraging costs (**c**, **d**). For the estimation of foraging costs, mean horizontal swimming speeds (BL s^{-1}) and turns>90° from the model results in Table 5-2 were implemented into a model relating spontaneous swimming costs to metabolism in sprat (Eq. 4). All calculations were based on estimates for fish feeding on either highly-evasive prey (copepods; *Acartia* spp.) or for fish feeding on non-evasive prey (*Artemia salina* in experiments, *Bosmina coregoni* for energy equivalents). See materials and methods section for further details on calculations

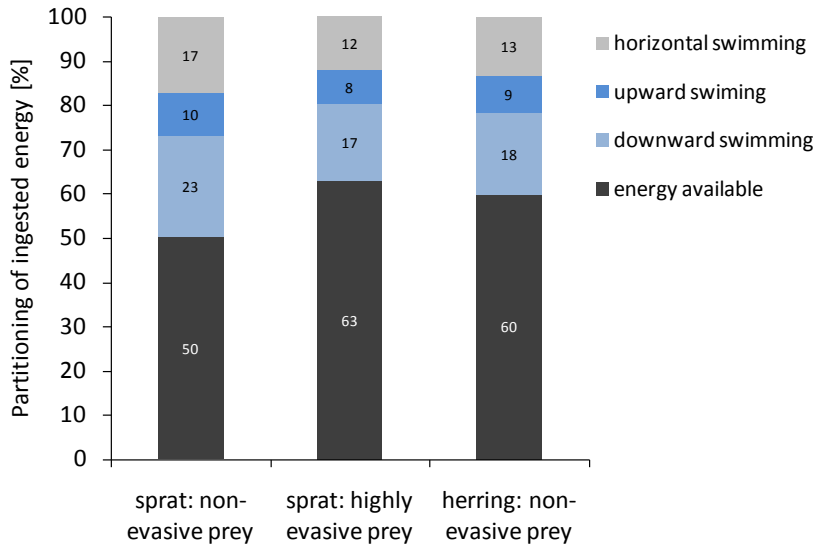

Figure 5-11 Energy partition for sprat and herring feeding on high prey concentrations (70 l^{-1}) of either non-evasive prey (*Artemia* nauplii in experiments, *Bosmina coregoni* for energy intake) or highly-evasive prey *(Acartia tonsa* in experiments, *Acartia clausii* for energy intake). See text for further details

5.4 Discussion

5.4.1 Limitations of the present methods

The intension of our tank design and experimental concept was to investigate feeding rates and related swimming patterns of undisturbed sprat and herring feeding on a wide range of prey concentrations. The large scale production of copepods was limited by the longer generation time of *A. tonsa* compared to *Artemia* nauplii so that we needed to limit the tank dimension of the experimental aquarium in space to allow establishing high prey concentrations (>100 l^{-1}) with *A. tonsa* C5 and adults for at least 60 min. However, despite the limited space provided all fish showed a natural feeding behaviour (Brachvogel *et al.* 2012) and there was no indication of tank effects on the swimming behaviour of fish.

We used constant temperatures and light conditions during experiments excluding the influence of changing environmental factors on the swimming behaviour of fish. However, salinity was different between sprat and herring experiments. Although salinity does not seem to influence swimming activities of sprat and herring directly (Turnpenny 1983), it may change the buoyancy and behaviour of the prey organisms used in our study. In herring experiments salinity was ~30 PSU, which is higher than salinities of *A. tonsa* rearing (18 PSU), but similar to salinities for *Artemia* rearing (33 PSU). Thus, copepods were possibly affected by this rapid increase in salinity in herring experiments which could result in less pronounced escape responses. This may partly explain why herring achieved higher biting rates in experiments with copepods than sprat (Brachvogel *et al.* 2012) but this difference in biting rates between the two fishes was observed only at low copepod concentrations (<40 l^{-1}). In addition, herring had like sprat lower feeding rates when preying on *A. tonsa* instead of *Artemia* and both fishes showed an S-shaped body curvation during feeding on *A. tonsa*. This indicates that copepods were similar evasive in both salinities. In sprat experiments with a salinity of 16 PSU, we can assume that *A. tonsa* did not behave differently to their natural behaviour as they were pre-acclimated to similar conditions. *Artemia* nauplii are generally tolerant to changes in salinity (Soundarapandian and Saravanakumar 2009) and are non-evasive under all circumstances, so that the lower salinities in sprat experiments are unlikely to make any differences compared to herring experiments with regard to the behaviour of brine shrimp nauplii. In conclusion, we cannot finally exclude the possibility that copepods were negatively affected by changes in salinity during experiments with herring, but they were in either case still more evasive than *Artemia* nauplii.

5.4.2 Fish behaviour

Fish in our experiments selected their prey visually during upward swimming when prey particles were visible against the light, similar to the behaviour reported for larval herring during particulate feeding (Rosenthal 1969; Munk 1992). Sprat is in general not able

to filter-feed (Bernreuther 2007; Brachvogel *et al.* 2012) and juvenile herring prey mainly by biting as they are fully able to filter feed not until a length of ~13 cm (Gibson and Ezzi 1985; 1990; 1992). Herring in the present study were, however, occasionally filter- feeding or gulping at concentrations >50 l^{-1}, but this behaviour was hard to detect on the underwater camera images and particulate feeding was the dominant feeding mode in both species even at higher prey concentrations (Brachvogel *et al.* 2012). We therefore assume that the detected swimming patterns are representative for particulate feeding in both fishes.

Particulate-feeding fish reduce their school density during feeding (van der Lingen 1994; Robinson 1995; Macy *et al.* 1998) in order to reduce the overlap of individual visual fields and to expanse the foraging efficiency (Eggers 1976; Duffy and Wissel 1988; Robinson and Pitcher 1989). This behaviour reflects an adaptive strategy in fish schools where individuals benefit from the protection against predators, but also have the particular problem that other school members are potential food competitors. A similar behaviour was observed in our experiments where both species swam in loose aggregations when no food was supplied, but changed to more individual swimming during feeding with higher distances between individuals.

5.4.3 Swimming patterns in relation to prey concentrations

Optimal foraging theory predicts how a predator should respond to changes in prey concentrations given the assumption that the optimal strategy maximizes the net rate of energy intake (Charnov 1976). There are in principal different possibilities to maximize the net energy intake for a fish during feeding, *e.g.* by minimizing the time spent for searching prey or minimizing the energy costs by adjusting the swimming speed in relation to prey concentration. For pelagic fishes, Ware (1975; 1978) developed a theoretical model of optimal foraging speed that predicts: as food concentration increases, fish increase their swimming speed up to an intermediate level of ~ 3 BL s^{-1}. The optimal foraging speed is defined as speed that maximizes the fish growth over time in relation to the net food intake per unit time, the standard metabolic rate and the costs of swimming, respectively. This intermediate speed of around 3 BL s^{-1} was likewise observed by Brett (1965) for maximum sustained speeds of sockeye salmon and is well in line with the presently found average overall swimming speeds between 2.92 and 3.39 BL s^{-1} (Table 5-3) when fish fed on higher prey concentrations (>40 l^{-1}). However, Ware's model predicts that to maximize the net energy intake, swimming speeds should progressively decrease with higher prey concentrations. The author stated that at higher prey concentrations the prey becomes too plentiful and the predator must spend a greater proportion of its time handling food. The predator can than reduce the cost of foraging by moving at a lower speed (Ware 1978). In the present study horizontal swimming speeds increased asymptotically with prey concentrations up to ~ 1 BL s^{-1} (Figure 5-3), but speeds were not clearly decreasing at higher zooplankton concentrations (>100 l^{-1}). Likewise were turning rates and accelerations not decreasing at higher prey concentrations. Only the downward vertical swimming speeds

decreased slightly at higher concentrations for sprat feeding on *Artemia* (Figure 5-9), but the standard deviations did not indicate any significant trends within the tested range of prey concentrations. The horizontal swimming speeds reached a plateau at concentrations between 30-39 l^{-1}, but biting rates were still increasing with prey concentrations (Brachvogel *et al.* 2012) and the asymptote of the maximum biting rate was not reached in any experiment. Thus, we did most likely not reach the prey concentrations were the handling time was limited by the high density of prey and a decrease in swimming speed would increase the net energy intake. However, this will be of relevance only in evinronments where fish can feed on plankton of very high aggregation, *e.g*. with more than 500 copepods l^{-1}.

5.4.4 Comparisons with other studies

Direct comparisons of feeding related swimming patterns between species are complicated because observations, analytical techniques and experimental designs differ widely. There are only a few studies about swimming patterns of zooplanktivorous particulate feeding fish at different prey concentrations. In some of these studies frequent turns and changes in direction were reported as common swimming patterns while feeding, *e.g.* for *Sardina pilchardus* (Garrido *et al.* 2007) or *Engraulis capensis* (James and Findlay 1989). However, turning rates in our study were determined differently than in the study by James and Findlay (1989) where changes in direction were defined as deviation of more than 20° from the motion recorded before. This motion was, however, recorded only for short periods of time (10-15 s) for single fish and only a few values at low prey densities were available (James and Findlay 1989). Sprat and herring in our study turned on average always more than 80°s^{-1} during feeding, whereby these direction changes were calculated as mean values of the fish group (n = 10) and averaged for a 10-min time interval. In the investigation by Garrido *et al.* (2007), turning rates were not quantified as functions of prey densities, so that we cannot directly compare our results with typical movements of other related species. Furthermore, the present study revealed that vertical swimming during particulate feeding of sprat and herring is of particular importance at higher prey concentrations (>20 l^{-1}), but in none of the cited studies vertical movements were described.

5.4.5 Prey type effects

Sprat and herring feeding on copepods showed an S-shaped curvation of their body directly before the attack (Figure 5-1), which was not observed when fish were feeding on *Artemia*. This behavioural difference was caused by the high escape capability of *A. tonsa* (Buskey 1994; Buskey and Hartline 2003) compared to *Artemia* nauplii (Robinson *et al.* 2007). A fast start of fish with an S-shaped bending of the body is typical of predator attacks from a standing start (Webb and Skadsen 1980). The S-shaped body posture allows a more balanced attack and avoids uncontrolled turning as the fish accelerates towards the prey

(Blake 2004). Swimming speeds and accelerations of an attack with an S-shaped body deformation depend on prey size, apparent prey size (angular size of prey in relation to retina size) and strike distance (Harper and Blake 1991). In the present study the S-shaped bending was not directly detected quantitatively from the images of the top camera, but the longer time for attacking copepods due to this body deformation was reflected in lower horizontal swimming speeds when fish fed on *A. tonsa* compared to fish feeding on *Artemia*. Likewise were the vertical speeds lower for sprat preying on copepods than for sprat feeding on *Artemia*, especially for the upward swimming when fish are biting (Figure 5-9). However, the asymptotic maximum values for mean turning rates ($°s^{-1}$) of sprat were similar for both prey types (Table 5-2). Likewise were the number of sharp turns (>90°) not different between the two prey types offered in both species. But in herring mean turning rates ($°s^{-1}$) were slightly lower during experiments with copepods in contrast to experiments with *Artemia* (Figure 5-3), which could be an effect of the lower range in prey concentrations tested for herring feeding on copepods compared to those feeding on *Artemia*. In conclusion, the different prey escape capabilities of brine shrimp nauplii and adult copepods were directly reflected in the feeding behaviour and resulted in lower swimming speeds and consumption rates when fish fed on highly evasive copepods compared to feeding on non-evasive *Artemia*.

5.4.6 Differences between sprat and herring

Juvenile sprat and herring form mixed schools in the shallow coastal regions of the North and Baltic Seas, whereby herring are on average 1-2 cm larger in size than sprat (Maes and Ollevier 2002). This size difference also occurred in our experiments and resulted in higher maximum horizontal swimming speeds (cm s^{-1}), but slightly lower absolute speeds (BL s^{-1}) in herring (max. 0.96 BL s^{-1}) than in sprat (max. 1.07 BL s^{-1}) when both species were preying on the same zooplankton type (Figure 5- 3, Table 5-2). However, this difference was only significant for the asymptotic maximum speed (BL s^{-1}) at higher prey concentrations (>30 l^{-1}) but not at lower prey concentrations as indicated by the identical parameter estimates for $ø_2$ and $ø_3$ (Eq. 3, Table 5-2). The mean vertical swimming speeds (BL s^{-1}) of herring were lower than in sprat when both species fed on *Artemia* nauplii (Figure 5-9). On the other hand, herring had higher mean turning rates ($°s^{-1}$) and acceleration values (cm s^{-2}) than sprat when both species were feeding on *Artemia* (Figure 5-7). This indicates that herring feeding on *Artemia* attacked prey closer to their mouth than sprat and thereby performed more sharp turns which can limit the simultaneously performed swimming speeds (BL s^{-1}). The faster speeds of sprat were likewise accompanied with lower rates of manoeuvrings (mean turning rates). When comparing the swimming patterns of both species during experiments with copepods, other conclusions might be possible: Herring had not only lower horizontal swimming speeds (BL s^{-1}) than sprat, but also lower maximum turning rates ($°s^{-1}$) and acceleration values than sprat (Figure 5-7). However, for a more conclusive interpretation of the energetic efficiency of feeding we need to compare the

metabolic costs while foraging in sprat and herring which is discussed in the following passage.

5.4.7 Field application of the data

Fish bioenergetics models are sensitive to assumptions regarding the magnitude and variability of activity rates, but so far swimming speeds in models for clupeoid fishes (*e.g.* Atlantic herring, Megrey *et al.* 2007; anchovy Politikos *et al.* 2011) are mainly modelled as functions of temperature and body mass, but not as functions of prey densities. Either a constant cruising speed is assumed (*e.g.* 2 BL s^{-1} for herring; Varpe and Fiksen 2010) or swimming speeds are considered to vary only with prey size or feeding mode (European anchovy; Politikos *et al.* 2011). Moreover, vertical swimming speeds are not considered in such models, but the present study revealed that vertical speeds contribute to more than 50% to the total swimming speed during particulate feeding of sprat and herring when prey concentrations are above 20 l^{-1} (Table 5-3). Thus, swimming speeds of particulate feeding fish should be based on measurements of both horizontal and vertical speeds when high prey concentrations (>20 l^{-1}) are assumed. Average copepod abundances in the field are, however, assumed to be rather low (< 10 l^{-1}; Colebrook 1979; Broekhuizen and McKenzie 1995), but might be higher in patches with more than 100 copepods l^{-1} (Folt and Burns 1999; Maes and Ollevier 2002). However, both sprat and herring feed selectively on larger, reproducing stages of copepods (Möllmann *et al.* 2003; Bernreuther 2007) and in zooplankton patches concentrations of prey with the preferred size will be most likely in the lower range of concentrations investigated in the present study (<20 l^{-1}). The analysis of swimming costs while feeding on such prey concentrations (Figure 5-10) revealed that herring have a clear energetic advantage above sprat when both species are preying on *Acartia* spp. (Figure 5-10 d). This is mainly caused by the higher biting rates (Brachvogel *et al.* 2012) in spite of lower swimming speeds in herring compared to sprat. However, when both species were feeding on non-evasive prey, sprat could achieve higher biting rates at these low concentrations but they were swimming relatively faster than herring so that the overall difference between sprat and herring is only marginal in terms of the net available energy (Figure 5-10 c). Energy costs during foraging were overall lower in both species during feeding on highly evasive-prey compared to feeding on non-evasive prey (Figure 5-10; Figure 5-11). This is basically an effect of the lower mean swimming speeds during feeding on copepods compared to fish feeding on *Artemia* (Figure 5-3) as the number of turns>90° were similar for both prey types and fish species, respectively (Figure 5-5). When considering only horizontal swimming patterns a copepod concentration of ~2 l^{-1} can cover the total energy costs during foraging in both fish species. However, the question remains if the S-shaped body curvation during feeding on copepods is excessively cost expensive and could therefore change the assumptions of lower energetic costs for feeding on evasive prey versus feeding on non-evasive organisms. Unfortunately, these S-shaped body deformations were not recognizable as turns and obviously not reflected in the variance in acceleration which could

be due to the relatively low image acquisition rate of 4 frames s^{-1}. At higher image acquisition rates one could possibly resolve the fast accelerations during the S-shaped attacks on copepods. In addition, metabolic rates have not been measured in sprat or herring during feeding on these two different prey groups, so that we cannot directly evaluate which feeding pattern is more cost expensive.

The analysis for fish feeding at a high prey concentration (70 l^{-1}) revealed that 50-63% of the ingested energy is left after metabolic requirements for total energy costs during foraging (including standard metabolism) are subtracted (Figure 5-11). As biting rates are still increasing with concentrations >70 l^{-1} but swimming speeds remain constant, the net available energy increases further with increasing prey concentration. For fish feeding on non-evasive prey, this would result in a net available energy at a concentration of 160 l^{-1} of 67 % of the consumed energy (438 J $fish^{-1}h^{-1}$) for herring and 59 % (371 J $fish^{-1}h^{-1}$) for sprat, respectively. For sprat feeding on copepods at a concentration of 160 l^{-1}, this would mean that 66% (369 J $fish^{-1}$ h^{-1}) of the total energy intake is left after metabolic demands during feeding were subtracted. This analysis further revealed that sprat need a prey concentration of ~14 l^{-1} when feeding on copepods in order to cover the energy demands for the total foraging costs (including vertical swimming). For herring, horizontal swimming patterns were observed only for experiments with *Artemia*. The resulting approximation of foraging costs at a high cladoceran concentration suggests that herring need a concentration of ~ 13 l^{-1} to cover the total mmetabolic energy demands while foraging (Figure 5-11). This is in accordance with the findings of the present study, that fish showed only slight movements in the vertical plane at lower prey concentrations (<15l^{-1}). Thus, the present results demonstrate that herring and sprat change their feeding behaviour with increasing prey concentrations. At low concentrations (below ~15 l^{-1}) mainly horizontal swimming patterns were observed (see also Brachvogel *et al.* 2012) and foraging costs at these low concentrations can be adequately determined by the combination of the present models for horizontal swimming speeds and numbers of turns>90° (Table 5-2) with a metabolic rate model for spontaneous swimming costs (Manuscript 2). At higher prey concentrations (>20 l^{-1}) both fishes showed considerable vertical swimming speeds so that estimates of foraging costs at these higher concentrations should be based on both horizontal and vertical swimming patterns. Furthermore, herring appear to have a clear feeding advantage above sprat when both species have a similar size typically observed in mixed schools. Future studies should investigate the occurrence of the presently found foraging related swimming patterns in the natural environment, *e.g.* in the shallow coastal areas of the Baltic Sea where schools of sprat and herring can be observed by 2D-undertwater-camera systems.

5.5 Acknowledgements

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6 General conclusions and outlook

The present thesis investigated metabolic rates and feeding behaviour of sprat (and partly herring) in order to contribute to the development of a specific bioenergetic model for sprat. On the one hand respirometry experiments were conducted in order to determine the effects of body mass, temperature and spontaneous activity on metabolic rates in sprat and on the other hand feeding experiments were undertaken to determine the type of functional response and feeding related swimming patterns in juvenile sprat and herring.

6.1 Respirometry experiments

Sprat lives in habitats with strong seasonal fluctuations in water temperature, so that accurate parameterisations of the temperature effect on metabolism is essential to construct a reliable bioenergetic model for this species. The results from the present study (Manuscript 1) demonstrate that temperature has a significant effect on the metabolic rate of sprat, resulting in a Q_{10} of 2.2 for both standard and routine metabolism over the temperature range from 9-21°C. This value is similar to that found in other clupeoids when respirometry experiments were conducted (Q_{10}= 1.8-2.2) and is similar to the value found in juvenile herring over temperatures between 9.7-14.2°C determined from starvation experiments (Bernreuther 2007). The results from the respirometry experiments further revealed that the original definition of standard metabolism (R_S) as metabolic rate at zero activity does not apply in sprat. R_S in sprat should be better defined as the nearest approximation of a metabolic rate at the lowest obtainable swimming activities, also termed as *fasting metabolic rate* by Jobling (1994). The method of percentile analysis (Herrmann and Enders 2000) to distinguish between standard and routine metabolism (R_R) revealed in many experiments very similar rates for both metabolic level. However, in some cases deviations occurred of up to 38%, suggesting that routine rates are less consistent and in bioenergetics budgets the R_S estimates should be used. The overall analysis of both temperature and body mass effects on standard metabolism resulted in a weight scaling exponent of b=1.073 (Manuscript 1), which is substantially higher than the majority of weight scaling exponents for fish reported in the literature (b ~0.8, Winberg 1960; Brett and Groves 1979; Jobling 1994). However, b measured for active fishes is significantly greater than b measured for animals at rest and is often close to 1 (Glazier 2009). The near isometric scaling of metabolism in active animals is mainly explained by the influence of the volume-related muscular power production on maximal metabolic rates (MLB-hypothesis; Glazier 2005; 2009). This means that in active animals b is predicted to be positively related to body length because as activity increases, an animal's metabolism becomes increasingly devoted to muscular energy expenditure, which scales in direct proportion to muscle mass, which in turn scales as body mass[1] (Calder 1984; Glazier 2005). Thus, it can be concluded that the permanently elevated activity level is the most likely reason for the high metabolic scaling

exponent in sprat. The isometric metabolic scaling should be taken into account in bioenergetics budgets for sprat, which were so far largely relying on parameter estimates derived from other clupeoids and partly on non-related species (Rudstam 1988; Arrhenius 1998).

The metabolic rates presented in Manuscript 1 included an unknown proportion of energy costs associated with spontaneous swimming. In order quantify such costs in sprat, simultaneous video recordings of swimming fish during some of the respirometry experiments were analysed (Manuscript 2). The purpose was to utilize measurements from acclimation periods of experiments where swimming patterns and oxygen consumption rates (MO_2; mgO_2 $fish^{-1}$ h^{-1}) were more variable than during the later routine phases, which were used to determine R_S and R_R. This new approach revealed that the elevated metabolic level during the acclimation phase above routine level is largely related to swimming activities. Overall, spontaneous swimming in sprat was characterized by low mean swimming speeds (0.28 - 0.74 BL s^{-1}) and high numbers of turns>90° (0.414 - 2.321 $fish^{-1}s^{-1}$), which is in accordance with previous descriptions on spontaneous swimming in various other fishes where frequent turns and low swimming speeds were reported (*e.g.* Blake 1983; Videler 1993; Tudorache *et al.* 2009). The final model to relate MO_2 to swimming speeds and numbers of turns>90° explained 68% of the variability in oxygen consumption rates and revealed that spontaneous swimming was 1.2-1.4-times the costs associated with standard metabolism, depending on temperature. This is in line with previous calculations by Sirois and Boisclair (1995) for "spontaneous activity metabolism" of brook trout (*Salvelinus fontinalis*), which was 1.0-1.4-times the costs associated with standard metabolism. The swimming speeds during the respirometry experiments were not affected by temperatures between 10 and 19°C (Manuscript 2) which is in conclusion with assumptions made in bioenergetic models for clupeoids: Rudstam (1988) assumed in a bioenergetic model for Atlantic herring that swimming speeds will decrease with temperatures only below 9°C and for European anchovy this threshold was set at 12°C in Politikos *et al.* (2011). Thus, swimming speed in sprat might decrease as well when temperatures are lower than in the present study. Since adult sprat commonly occupy water layers with temperatures down to 5°C in the Baltic Sea (Stepputtis 2006), it would be of great interest to investigate the effect of temperature on both metabolism and swimming speeds at lower temperatures, *e.g.* between 5 and 9°C. However, although swimming speed was not directly affected by water temperature, metabolic costs during spontaneous swimming showed a greater increase with swimming speed at increasing temperatures. This indicates that the scope for growth (net energy gain) in sprat may decline at temperatures above 19°C where gastric evacuation rates and hence maximum consumption rates decrease (Bernreuther *et al.* 2009) but swimming costs will still increase. However, this study (Manuscript 2) clearly has some limitations as the experiments were not specifically designed to analyse swimming costs in feeding fish. The model allows to quantify energy costs for spontaneous swimming and should be applied to similar movement patterns and not extrapolated to much higher swimming speeds.

6.2 Feeding experiments

Previous laboratory investigations on the feeding behaviour of clupeids did not confirm the type of functional response during particulate feeding as in most studies low prey concentrations were not tested systematically, *e.g.* Gibson and Ezzi (1985) examined the feeding behaviour in herring mainly at high prey concentrations of up to 1000 l^{-1} where the type of functional response was not visible. The present study on the functional response of juvenile sprat and herring (Manuscript 3) filled this gap by testing biting rates of fish feeding on small scaled prey concentrations from 1-160 l^{-1}. Both clupeids showed a type-II functional response for two prey types with different escape capabilities (*Artemia* nauplii and *Acartia tonsa* C5-adults). In a type-II response, the number of prey consumed per time increases with increasing prey concentration but the rate of increase is progressively reduced until an asymptote is reached. The obtained maximum biting rates of sprat and herring (~2 s^{-1} for *Artemia* nauplii and ~1 s^{-1} for copepods) support field observations on rapid depletion of local zooplankton patches by these two clupeids (Maes *et al.* 2005). For both predators feeding rates were significantly higher with *Artemia* nauplii than with *A. tonsa*. The lower feeding rates of both predators with *A. tonsa* are assumed to be mainly caused by the well-developed escape response of copepods compared to *Artemia* (Trager *et al.* 1994; Kiørboe 2010). Thus, the handling time was significantly longer when fish fed on copepods as they were showing an *S*-shaped deformation of the body before attacking a copepod. The relative contribution of handling time to total feeding time increased and ultimately limited the number of consumed prey in a given time. This study further revealed that sprat strictly sticks to particulate feeding, even at prey concentrations >100 l^{-1}. It has been reported earlier that sprat is an obligate particulate feeder by Bernreuther (2007), but this has not been tested systematically before. Likewise, it remained unknown at which size herring start to filter-feed as the development of this feeding mode is likely to be a continuous process during ontogeny. Early juveniles exhibit only particulate feeding and fish at a length of 13-20 cm are fully able to filter-feed (Gibson and Ezzi 1985; 1990). In the present experiments, herring (~7 cm TL) were occasionally filter-feeding (or gulping) when prey concentrations were >50 l^{-1}, but the dominant feeding mode was particulate feeding for all prey concentrations. Hence, it can be concluded that juvenile herring exhibit mainly particulate feeding and are directly competing for the same food recourses as similar sized sprat. In conclusion, the present study confirmed that sprat and herring show a type-II functional response. It was further indicated that limited food environments may favor herring over sprat as herring achieved higher biting rates than sprat at low copepod concentrations (<40 l^{-1}). The parameterization for two relevant prey types (evasive and non-evasive) will improve bioenergetic models which can be incorporated in end-to-end ecosystem models like in the NEMURO-model framework (Megrey *et al.* 2007) or ATLANTIS (Fulton *et al.* 2004).

Another goal of these feeding experiments was to determine feeding related swimming patterns of sprat and herring (Manuscript 4) in order to allow modelling activity as

a function of prey biomass in bioenergetic and foraging models. This can improve fits between observed food consumption rates and those predicted by a fish bioenergetic model. The use of images from a top-camera allowed to detect horizontal swimming patterns, which were dominant during feeding on low prey concentrations (<~20 l^{-1}), whereas at higher prey concentrations (40-160 l^{-1}) vertical swimming (observed by an underwater camera) contributed significantly to the overall swimming speeds. The horizontal swimming patterns, such as mean swimming speeds (BL s^{-1}; cm s^{-1}), mean turning rates (° s^{-1}), variance in accelerations (cm s^{-2}) and numbers of turns>90° ($fish^{-1}s^{-1}$) were modelled as asymptotic increasing functions (Gompertz function) of prey concentrations (l^{-1}) for both fish species and two prey types, respectively. Horizontal swimming speeds during feeding were up 2.1-times higher than the swimming speeds during routine swimming (non-feeding) and were overall lower for fish feeding on copepods compared to those feeding on *Artemia* nauplii. The observed mean swimming speeds during non-feeding phases for both sprat and herring (mean = 0.51 BLs^{-1}) were similar to routine swimming speeds observed for sprat in the respirometer chamber (mean = 0.57 BL s^{-1}), although in the feeding experiments fish were swimming in a much larger tank (~239 l) than during the respirometry experiments (~80 l). From this one can conclude that speeds of ~0.5-0.6 BL s^{-1} are typical for both sprat and herring during routine swimming.

It was assumed that energy costs of sharp turns (>90°) are of particular interest to assess the costs for feeding in sprat. However, the analysis of swimming patterns during foraging (Manuscript 4) revealed that the number of turns >90° was not very consistent and did only roughly follow an increasing curve with increasing prey concentrations. Moreover, the maximum number of turns>90° of 0.367 $fish^{-1}$ s^{-1} in the feeding experiments was much lower than during the respirometry experiments with a mean value of 1.07 $fish^{-1}s^{-1}$ (Manuscript 2). This is most likely an effect of the limiting size of the respirometer chamber which probably artifically increases the spontaneous activity level by forcing frequently high degrees of turns (*e.g.* Boisclair and Tang 1993; Tang *et al.* 2000). Mean turning rates ($°s^{-1}$) were, however, more suitable as estimator of manoeuvrings in relation to prey concentrations in the feeding experiments, but were not positively correlated with mean oxygen consumption rates in respirometry experiments due to the long measuring intervals of 23 min. However, for first approximations of foraging costs one can use the numbers of turns>90° observed during the feeding experiments (Manuscript 4).

There was no significant difference in the feeding rates between both predators, despite at low concentrations of copepods (<40 l^{-1}) where herring could achieve higher biting rates than sprat. This indicated a possible feeding advantage of herring above sprat, which was likewise confirmed by the inspection of the feeding associated swimming patterns and foraging related energy costs. In order to make assumptions on the energy costs associated with feeding, the mean swimming speeds and numbers of turns>90° from the feeding experiments (Manuscript 4) were implemented into the model for spontaneous swimming costs presented in Manuscript 2. The model application, however, resulted in unreasonable high MO_2 values when swimming speeds far beyond the measured range (0.28-0.74 BL s^{-1})

were inserted. This is mainly caused by the fact that the measured range of swimming speeds during the respirometry experiments was very narrow and there was a strong interaction between temperature and costs for swimming. Thus, the increase in MO_2 with swimming speed was very high and resulted in a swimming speed exponent of v = 3.28 (Manuscript 2). This model was therefore only used to estimate for the cost of horizontal swimming speeds and number of turns, which were similarly low as in the respirometry study. This approach revealed that herring have a clear energetic advantage above sprat when both species are preying on copepods at lower concentrations (<20 l^{-1}). This is mainly caused by the higher biting rates (= higher energy intake rates), but lower relative swimming speeds (BL s^{-1}) of herring compared to sprat. It was further shown that there is no clear difference in the energetic efficiency of feeding between both species when fish are preying on low concentrations (<20 l^{-1}) of non-evasive organisms, such as cladocerans. At higher prey concentrations (>20 l^{-1}) both species showed considerable vertical swimming speeds which resulted in much higher overall speeds (~3 BL s^{-1}) than the maximum horizontal speeds alone (~1 BL s^{-1}). These vertical speeds can be divided into two components, one representing the vertical upward swimming where fish were biting (see Manuscript 3) and swimming with speeds ~ 1.2 BL s^{-1}. The other component represents the vertical downward bursts with speeds ~ 5.5 BL s^{-1}. The downward swimming accounted on average for 20% of the total time needed for one complete vertical swimming pattern during feeding. For each of these two components, foraging costs were estimated separately by the application of a modified metabolic rate model where turns>90° were excluded and the swimming speed exponent was estimated again from the original swimming speed-MO_2 values at 16°C presented in Manuscript 2 (see also Material and Methods in Manuscript 4). This model allowed a more realistic approximation of metabolic costs at higher swimming speeds than the original model where temperature effects on swimming costs and turns>90° were included (see Manuscript 2). The costs for vertical swimming speeds and those obtained for the horizontal speeds were combined in order to approximate for the total metabolic costs during feeding at high prey concentrations (*e.g.* at 70 l^{-1}). This approach revealed that sprat would need a copepod concentration ~ 14 l^{-1} in order to cover energy demands for swimming (including fast vertical speeds and R_S). This value is fully in agreement with the observation that sprat conduct considerable vertical swimming only at higher concentrations above 15-20 l^{-1}. Likewise did herring show considerable vertical swimming only at concentrations >20 l^{-1} and the model application for energy costs during foraging with fast vertical swimming speeds suggests that they would need a concentration of ~ 13 cladocerans l^{-1} to cover the total energy demands during foraging. The inspection of the overall swimming costs (including costs for vertical swimming) together with the estimated energy intake rates based on the respective biting rates (from Manuscript 3) suggests that herring also have an energetic advantage when both species are preying on high prey concentrations of non-evasive prey. For example at a concentration of 70 cladocerans l^{-1} herring would overall invest 40% of the total ingested energy into foraging (vertical and horizontal movements + R_S) whereas sprat would spend 50% of the energy intake for swimming and maintenance metabolism. As biting

rates continue to increase above concentrations of 70 l^{-1} (Manuscript 3), but swimming speeds remained constant above 30-39 l^{-1} (Manuscript 4), the net energy available will also further increase with prey concentration in both species. The lower swimming speeds of fish feeding on highly-evasive prey compared to those feeding on non-evasive prey lead to lower foraging costs for fish feeding on *Acartia* compared to fish feeding on *Artemia*. However, the question remains if the S-shaped body deformation during feeding on copepods is excessively cost expensive and could therefore change the assumptions on lower energetic costs for feeding on evasive prey versus feeding on non-evasive organisms. As we have no measurements on metabolic rates of sprat or herring during feeding we cannot make final conclusion about the energetic efficiency of feeding on evasive versus non-evasive prey.

Concluding, the results presented in Manuscript 3 and 4 demonstrate that sprat and herring have different feeding behaviours depending on prey concentrations: at low concentrations (<15 l^{-1}) fish swim mainly in the horizontal plane with only slight vertical movements and at higher prey concentrations fish show fast vertical swimming speeds. Consequently, foraging costs need to be determined differently for the two feeding behaviours which are accompanied by different swimming modes: at prey concentrations below ~15 l^{-1} one can use the obtained models for horizontal swimming patterns from Manuscript 4 in combination with the corresponding biting rates (Manuscript 3) and the MO_2-model presented in Manuscript 2. At higher prey concentrations the elevated vertical swimming speeds should be taken into account, but for approximations of foraging costs a MO_2- swimming speed model should be used which is based on measurements at similar high swimming speeds. Thus, in future studies one should investigate metabolic rates of sprat at a wider range of swimming speeds for more reliable conclusions on foraging costs during feeding in plankton patches with higher concentrations.

6.3 Outlook

The present study investigated the effects of temperature, body mass and spontaneous swimming activities on sprat metabolic rates. However, some other metabolic processes need further investigations. For instance, measurements on feeding related metabolic rates (SDA-effects) at different ration levels, temperatures and fish body masses should be conducted. A first step could be to measure the overall “feeding metabolism” (Jobling 1994) including both costs for digesting the prey and costs produced by handling and ingesting prey. Although it is now possible to estimate the costs of spontaneous swimming in sprat (Manuscript 2), no final assumptions on the overall foraging costs or optimal swimming speeds can be made. For the calculation of optimal swimming speeds, further knowledge on energy costs of different swimming modes is required. Burst-and-glide swimming can be more efficient than steady continuous swimming (Ware 1978; Videler and Weihs 1983) and seems to be a typical swimming mode in clupeid fishes (Blake 1983; Videler 1993), probably when they are searching for food. Thus, respirometry experiments should be conducted where sprat can show a wider range of swimming speeds. This would allow

calculating daily rations and growth rates for different modes of swimming such as spontaneous swimming, feeding activity, burst-and-glide swimming or continuous straight-line swimming.

In order to validate the results on feeding related swimming patterns presented in this thesis, one needs to investigate the feeding behaviour of sprat and herring in the field. Therefore, 2D observations from a camera positioned below a fish school can give reasonable measurements of total swimming speeds. The results presented in Manuscript 4 allow relating horizontal to vertical swimming speeds and therefore to approximate for overall swimming speeds when only 2D-measurements are available. Since sprat and herring feed selectively on larger, reproducing individuals (Casini *et al.* 2004), one should further extend the present results on functional response curves by measurements of feeding rates in relation to prey sizes. In addition, feeding experiments should be conducted with fish of different body masses, temperatures and light levels in order to complete the estimations of functional response curves.

In general temperature effects on metabolic rates and swimming speeds are investigated with pre-acclimated animals held at a constant temperature. Likewise were all metabolic rates and swimming patterns in the present study measured with fish acclimated to experimental conditions (temperature and tank size). However, sprat and herring both show diel vertical migration patterns (Blaxter and Holliday 1963; Nilsson *et al.* 2003; Stepputtis 2006) which are associated with rapid changes in temperature, salinity and light level. In spring and early summer in the central Baltic Sea clupeids stay below the halocline during the day and near the surface during the night (Gröhsler *et al.* 2000; Szczucka 2000; Stepputtis 2006). It has been suggested that fish with cyclic temperature changes grow faster than those held at constant temperatures but more recent studies revealed that DVM is most likely a multi-faceted process driven by an interplay between bioenergetics benefits and predator avoidance (Mehner *et al.* 2011; Mehner 2012). A systematic investigation on the effect of changing temperatures on metabolism could answer questions about the bioenergetics efficiency of diel vertical migration (DVM) in sprat and herring.

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Individual scientific contributions to the multiple-author manuscripts and outline of publications

Manuscript 1: Effects of temperature and body mass on metabolic rates of sprat, *Sprattus sprattus* L. – published in *Marine Biology* (2010): 157: 1917-1927

All statistical analyses and text writing was done by Laura Meskendahl under supervision of Professor Dr. Axel Temming, who critically reviewed the manuscript and gave valuable comments. All experiments were performed by Laura Meskendahl under supervision of Jens-Peter Herrmann, who was involved in elaborating the concept for this study, designing the respirometer chamber and handling with data acquisition systems and programs.

Manuscript 2: Energy costs of spontaneous swimming in sprat, *Sprattus sprattus* L. at different water temperatures – re-submitted to *Marine Biology* in 2012 (*under review*)

This paper has been re-submitted to *Marine Biology* (Springer Science and Business Media) in 2012 after revision. No final decision was made until this thesis was finished.
Experiments were conducted by Laura Meskendahl under supervision of Jens-Peter Herrmann, who was involved in the development of the experimental design and respirometer system. The fish-tracking program was developed by René Pascal Fontes from the HAW Hamburg (University of Applied Sciences) based on specifications by Laura Meskendahl and Jens-Peter Herrmann. All text writing and statistical analysis was conducted by Laura Meskendahl under supervision of Professor Dr. Axel Temming, who critically reviewed the manuscript.

Manuscript 3: Functional responses of juvenile herring and sprat in relation to different prey types – published in *Marine Biology* 2012 (*in press*), online published 03 November 2012 – DOI 10.1007/s00227-012-2104-5

The text writing, statistical analyses and graphical presentations were conducted by Rini Brachvogel with contributions by Laura Meskendahl and under supervision of Professor Dr. Axel Temming. All experiments were conducted in equal parts by Laura Meskendahl and Rini Brachvogel under the supervision of Jens-Peter Herrmann, who helped with the technical realization and was involved in the conceptual design of this study.

Manuscript 4: Swimming patterns of juvenile sprat and herring in relation to prey concentration and type –*not published*

All experiments were conducted in equal parts by Laura Meskendahl and Rini Brachvogel under the supervision of Jens-Peter Herrmann. The analysis of swimming patterns and all calculations were done by Laura Meskendahl. All text writing, statistical analysis and graphical presentation was undertaken by Laura Meskendahl under supervision of Professor Dr. Axel Temming, who critically reviewed the manuscript and gave valuable comments.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift „Metabolic rates and feeding behaviour of sprat, *Sprattus sprattus* L.“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Hamburg, November 2012 Laura Meskendahl