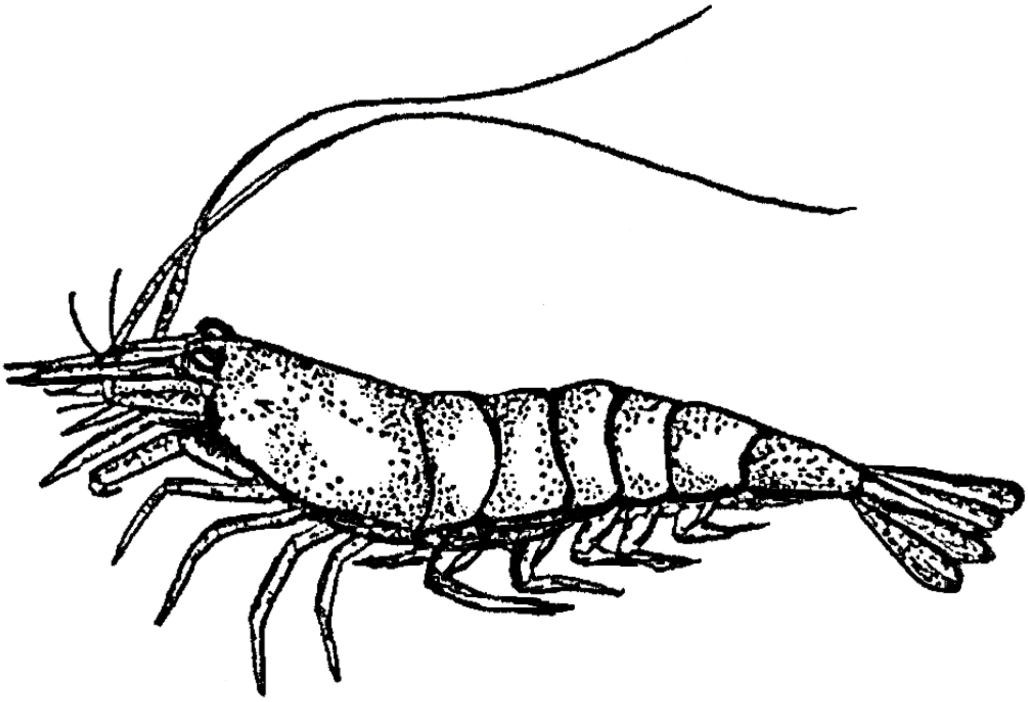
Growth in common brown shrimp *Crangon crangon* (Caridea, L. 1758). Improving growth parameterization in future lifecycle models



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1

Abstract

The common brown shrimp (*Crangon crangon*) is a species of high economical and ecological value in the southern North Sea ecosystem. Its presence in almost all areas, not only makes *C. crangon* a key species by being both predator and prey for a wide variety of other ecosystem inhabitants, but also forms the basis of a 500-vessel strong fishery that lands up to 40,000 tons annually, generating revenues of around 120 million euros. Due to the lack of moult enduring hard structures, a year-round lasting spawning period and the short-lived nature of the species, age determination in the common brown shrimp is nearly impossible. Hence, common-, age-based management approaches, such as the maximum sustainable yield (MSY) were found not applicable for the common brown shrimp stock. Aiming at the certification of the fishery by the marine stewardship council (MSC), in 2016 a self-management plan was introduced, in which measures alternative to a quota were set. The potential benefits and effects of these measures were calculated using a species-specific yield per recruit model, parameterized with growth data from decades of growth research on the species. The results of this model and the conclusions drawn for the management are highly influenced by the given growth input. Although the species, due to its extensive use, has been the subject of growth research since the late 1940th, to date questions remain about the factors influencing the growth in common brown shrimp. The aim of this thesis is to address some of these questions, and to further improve the parameterization in future growth calculations.

Crucial for the survival and growth of common brown shrimp is known to be food availability. At the same time, parts of the common brown shrimp population are-, depending on season, known to be exposed to prolonged periods of starvation. Despite their omnivorous feeding behaviour and the otherwise very productive ecosystem, in winter up to 80 percent of the population can be in a starving condition. The response of C. crangon to starvation has studied previously, but no study has been conducted in which the animals were starved for long periods and then fed again. **Manuscript 1** addresses the influence of prolonged food deprivation followed by re-feeding on the growth of the common brown shrimp. In laboratory experiments, animals were deprived of food for different periods of time and were re-fed thereafter. Subsequently, the moulting interval and the growth increment, determined individually, were compared with a control group that was fed ad libitum for the entire trial period. Food deprivation significantly increased the time between two successive moults and reduced the growth increment into a negative range. Shrinkage had previously been observed in the common brown shrimp occasionally but was first observed systematically in manuscript 1. In addition to the prolongation of the moulting interval during food deprivation, which was previously considered to mainly be a function of temperature and size, shrinkage was identified as a physiological necessity to compensate for the dry mass lost during the starvation period. The results from manuscript 1, combined with the observations of other authors that especially in winter up to 80 % of the population show a condition that indicate prolonged starvation, could be used to adjust growth predictions of the stock in winter. Since egg deposition and egg laying are linked to a moult event, the delayed moult due to starvation could affect the reproductive cycle of starving females. Reduced growth during starvation, and especially shrinkage could reduce catch quantities in spring fisheries, after winter with food scarcity.

In addition to gaining a better understanding of the influence of starvation and re-feeding on growth, in **Manuscript 2** the effects of in field density, a factor that could potentially trigger starvation in the shrimp stock, was investigated. Conserving the stock due to management measures, especially when reducing effort or increasing mesh size, leads to a local increase of individuals and hence density. Concerns towards density dependent growth counteracting potential benefits of a newly implemented measures, showed the need of further investigations into shrimp growth at different densities. In **Manuscript 2**, three different approaches, in which different proxies for growth were used, to compare growth potential in common brown shrimp caught at different densities. Besides laboratory growth observations and dry weight conditions from field samples the relative abundance of two successive length classes in samples from the demersal young fish survey (DYFS) were used to detect a potential decrease in growth, with increasing density. Growth proxies at different densities were compared within a short period of time (30-50 days), within month of one summer and between the same months in different years. In none of the three approaches indications for a potential growth limitation with increasing density was found. In summer, when the expected effects of a conserving measure are greatest, no signs for poorer growth at higher densities was found. On the contrary, animals sampled at the highest observed density usually showed the best growth potential. This led to the conclusion that some of the main factors determining population size are bottom up driven. Hence, do good conditions lead to high densities, rather than high densities tend to worsen the shrimps' conditions. The fact that no deterioration in growth performance was observed at higher densities in any of the approaches, as well as the fact that the density in the field fluctuates far more naturally, than it would with a sparing measure, led to the conclusion that concerns about loss of profit with density limitation are unjustified.

While density did not serve as an explanation for any of the observed growth differences between different growth trials, the seasonal origin of the sampled individuals was found valid to do so. During the analysis of the laboratory experiments in the context of density limitation of growth, there was a striking pattern towards particularly well growing animals in different length classes, recognisable through the season. In 2011, Hufnagl and Temming published laboratory experiments that pointed to a so-called cohort effect. Animals of the same length grew very differently, which was attributed to the fact that they were caught five weeks apart, and belonged to two different cohorts. Since different growth rates within the season would have an impact on the calculations to determine stock development, the focus of **Manuscript 3** was on the investigation of the so called "Cohort Effect". Growth potential, both as a direct measure from laboratory experiments and derived from a set of dry weight condition data was compared for individuals of the common brown shrimp (Size 20-70 mm), and during the season of different years between 2006 and 2021. Both the growth experiments as well as the analysis of dry weight data, pointed at a specific cohort being consistently responsible for the highest growth observations throughout the season. Larger increments as well as shorter moulting intervals at the same experimental conditions, were found in individuals that were supposedly hatched from winter eggs. The cohort effect was observed to be stronger in some years of the time series than in others. Especially in 2019, which was also an exceptional year from the fishery point of view, no growth differences were observed within the season. The enormous shrimp stock in 2018, as well as a relatively cold winter in 2018/19, were identified as potential reasons for the absence of the cohort effect in 2019. Implications for growth calculations were highlighted, and the protection of specifically the well-growing cohort was identified as a possible management objective.

In **Manuscript 4** the development of a method to determine moult interval in common brown shrimp, from frozen field samples, was intended to allow in situ growth rate without the necessity of costly and elaborate growth experiments. Besides growth increment, the actual increase in length, moulting interval determines the frequency at which the length increase occurs. Hence knowing the period between two successive moults, is essential for growth rate calculations. Since the determination of moulting interval requires time consuming and elaborate laboratory experiments, and the results are error prone to the housing condition, the need for a method of in situ moulting interval determination from field samples is high. This is also the reason, why a large part of the observed moulting intervals was not measured individually but calculated on the basis of animals moulting within a short period of time. Based on this principle, the moult interval for a given length class should be calculated using recently moulted animals within a field sample. To identify the freshly skinned animals, a device was developed that could empirically determine the carapace hardness using a method for hardness determination known from materials science. By validating the device with moulting observed in the laboratory, a method was developed based on which it is possible to identify a recent skinning by means of carapace hardness and dry weight.

Zusammenfassung

Die Nordseegarnele (Crangon crangon) ist eine Art von hohem wirtschaftlichem und ökologischem Wert im Ökosystem der südlichen Nordsee. Ihr Vorkommen in fast allen Gebieten macht *C. crangon* nicht nur zu einer Schlüsselart, da sie sowohl Räuber als auch Beute für eine Vielzahl anderer Ökosystembewohner darstellt, sondern bildet auch die Grundlage für eine ca. 500 Fischereifahrzeuge umfassende Fischerei, die jährlich bis zu 40.000 Tonnen Garnelen anlandet und damit Einnahmen in Höhe von rund 120 Millionen Euro erzielt. Aufgrund des Fehlens die Häutung überdauernder Hartstrukturen, einer ganzjährig andauernden Laichzeit sowie der Kurzlebigkeit der Art, ist eine Altersbestimmung bei der Nordseegarnele nahezu unmöglich. Herkömmliche, altersbasierte Bewirtschaftungsansätze wie der Maximum Sustainable Yield (MSY) haben sich daher für den Nordseegarnelenbestand als nicht anwendbar erwiesen. Im Zuge der Zertifizierung der Fischerei durch den Marine Stewardship Council (MSC), wurde 2016 ein sogenannter Selbstmanagementplan impliziert, in dem alternative Maßnahmen zu einer Quote festgelegt wurden. Der potenzielle Nutzen und die Auswirkungen dieser Maßnahmen wurden mithilfe eines artspezifischen Yield per Recruits Modells berechnet, welches mit Wachstumsdaten aus jahrzehntelanger Wachstumsforschung an der Art parametrisiert wurde. Die Ergebnisse dieses Modells und die daraus gezogenen Schlussfolgerungen für eine mögliche Bewirtschaftungsstrategie des Bestandes, werden in hohem Maße durch den gegebenen Wachstumsinput beeinflusst. Obwohl die *C. crangon* aufgrund der extensiven Nutzung seit den späten 1940er Jahren Gegenstand intensiver wissenschaftlicher Untersuchungen ist, bleiben bis heute einige Fragen zum Wachstum der Art unbeantwortet. Ziel dieser Arbeit ist es, einige dieser Fragen zu klären und die Parametrisierung bei zukünftigen Wachstumsberechnungen so zu verbessern.

Entscheidend für das Überleben und Wachstum der Nordseegarnele ist bekanntermaßen die Verfügbarkeit von Nahrung. Gleichzeitig ist bekannt, dass Teile der Nordseegarnelenpopulation - je nach Jahreszeit - längeren Hungerperioden ausgesetzt sind. Trotz ihres omnivoren Fressverhaltens und des ansonsten sehr produktiven Ökosystems, in dem sich die Art aufhält, können sich im Winter bis zu 80 Prozent der Population in einem Zustand befinden, welcher auf die Überdauerung längerer Hungerperioden hindeutet. Die Reaktion von *C. crangon* auf Hunger wurde bereits an anderer Stelle untersucht, jedoch wurde bis heute keine Studie durchgeführt,

bei der die Tiere über einen längeren Zeitraum hungerten und anschließend wieder gefüttert wurden, was dem Ende einer im Feld auftretenden Nahrungsknappheit am nächsten kommen würde. **Manuskript 1** befasst sich mit dem Einfluss von längerem Nahrungsentzug und anschließender Wiederfütterung auf das Wachstum der Nordseegarnele. In Laborexperimenten wurde den Tieren für unterschiedlich andauernde Zeiträume die Nahrung entzogen und anschließend wurden die Tiere erneut ad-libitum gefüttert. Anschließend wurden das Häutungsintervall und der Wachstumszuwachs, welche individuell bestimmt wurden, mit einer Kontrollgruppe verglichen, die während des gesamten Versuchszeitraums ad-libitum gefüttert wurde. Durch den Nahrungsentzug verlängerte sich die Zeit zwischen zwei aufeinander folgenden Häutungen erheblich und der Längenzuwachs sank in den negativen Bereich. Schrumpfen war schon früher gelegentlich bei der Nordseegarnele beobachtet worden, wurde aber in Manuskript 1 erstmals systematisch beobachtet. Neben der Verlängerung des Häutungsintervalls-, welches bisher hauptsächlich als Funktion von Temperatur und Größe angesehen wurde, konnte das Schrumpfen als physiologische Notwendigkeit identifiziert werden, um für die während des Nahrungsentzuges verlorene Trockenmasse zu kompensieren. Die Ergebnisse aus Manuskript 1 in Verbindung mit den Beobachtungen anderer Autoren, dass insbesondere im Winter bis zu 80 % der Population einen Zustand aufweisen, der auf eine anhaltende Nahrungsknappheit hindeutet, könnten zur Anpassung der Wachstumsprognosen für den Bestand im Winter herangezogen werden. Da die Eiablage eng mit der Häutung der Tiere verknüpft ist, könnte die verzögerte Häutung den Fortpflanzungszyklus hungernder Weibchen beeinträchtigen. Verringertes Wachstum während einer Nahrungsknappheit und insbesondere das Schrumpfen könnten die Berechnung potenzieller Fangmengen in der Frühjahrsfischerei nach einem Winter mit Nahrungsknappheit verringern.

Zusätzlich zu einem besseren Verständnis des Einflusses von Hunger auf das Wachstum wurden in **Manuskript 2** die Auswirkungen der Dichte-, ein Faktor der möglicherweise Nahrungsknappheit im Garnelenbestand auslösen könnte, untersucht. Die Schonung des Bestands durch Bewirtschaftungsmaßnahmen, insbesondere durch die Verringerung des Fischereiaufwands oder die Vergrößerung der Maschenweite, führt zu einer lokalen Zunahme der Individuen und damit der Dichte. Bedenken hinsichtlich eines dichteabhängigen Wachstums, das dem potenziellen Nutzen einer neu eingeführten Maßnahme entgegenwirkt, zeigten, dass weitere Untersuchungen zum Wachstum der Garnelen bei unterschiedlichen Dichten erforderlich sind. In Manuskript 2 wurden drei verschiedene Ansätze verwendet, um das Wachstumspotenzial von Nordseegarnelen zu vergleichen, welche bei unterschiedlich hoher Dichte gefangen wurden. Neben Wachstumsbeobachtungen im Labor und Trockengewichts-bedingungen aus Feldproben wurde die relative Häufigkeit von zwei aufeinanderfolgenden Längenklassen in Proben aus der Demersal Young Fish Survey (DYFS) verwendet, um eine potenzielle Abnahme des Wachstums mit zunehmender Dichte festzustellen. Die Wachstumsindikatoren bei unterschiedlichen Dichten wurden innerhalb eines kurzen Zeitraums (30-50 Tage), innerhalb der Monate eines Sommers und zwischen denselben Monaten in verschiedenen Jahren verglichen. Bei keinem der drei Ansätze wurden Hinweise auf eine mögliche Wachstumsbegrenzung mit zunehmender Dichte gefunden. Im Sommer, wenn die erwarteten Auswirkungen einer den Bestand schonenden Maßnahme am größten sind, wurden keine Anzeichen für ein schlechteres Wachstum bei höheren Dichten gefunden. Im Gegenteil wiesen die Tiere, die bei der höchsten beobachteten Dichte beprobt wurden, in der Regel das beste Wachstumspotenzial auf. Dies lässt den Schluss zu, dass einige der wichtigsten Faktoren, die die Populationsgröße bestimmen, „Bottom-Up“ gesteuert sind. Gute Nahrungsbedingungen führen hohen Dichten, und nicht hohe Dichten zu schlechten Nahrungsbedingungen. Die Tatsache, dass bei keinem der Ansätze eine Verschlechterung der Wachstumsleistung bei höheren Dichten beobachtet wurde, sowie die Tatsache, dass die Dichte im Feld auf natürliche Art und Wiese deutlich stärker schwankt, als dies bei Einführung einer den Bestand schonenden Maßnahme der Fall wäre, führte zu der Schlussfolgerung, dass Bedenken hinsichtlich einer den Nutzen überdeckenden Dichtelimitierung, unberechtigt sind.

Während die Dichte nicht als Erklärung für die beobachteten Wachstumsunterschiede zwischen den verschiedenen Wachstumsversuchen diente, erwies sich die saisonale Herkunft der beprobten Individuen als stichhaltige Erklärung. Bei der Analyse der Laborexperimente im Zusammenhang mit der möglichen Dichtelimitierung des Wachstums wer ein auffälliges Muster hin zu besonders gut wachsenden Tieren, das über die Saison hinweg erkennbar blieb, zu beobachten. Im Jahr 2011 veröffentlichten Hufnagl und Temming Laborexperimente, die auf einen sogenannten Kohorteneffekt hinwiesen. Tiere gleicher Länge, die zu unterschiedlichen Zeitpunkten in der Saison gefangen wurden, wuchsen unter gleichen Bedingungen sehr unterschiedlich. Dies wurde darauf zurückgeführt, dass Tiere zu zwei verschiedenen Kohorten gehörten, welche zu unterschiedlichen Wachstumspotentialen fähig sind. Da sich unterschiedliche Wachstumsraten innerhalb der Saison auf die Berechnungen zur Bestimmung der Bestandsentwicklung auswirken könnten, lag der Schwerpunkt in **Manuskript 3** auf der

Untersuchung des so genannten "Kohorteneffekts". Das Wachstumspotenzial, sowohl als direktes Maß aus Laborexperimenten als auch abgeleitet aus einem Satz von Trockengewichtskonditions-Daten, wurde für Individuen der Nordseegarnele (Größe 20-70 mm) und innerhalb der Saison verschiedener Jahre zwischen 2006 und 2021 verglichen. Sowohl die Wachstumsexperimente als auch die Analyse der Trockengewichtsdaten deuteten darauf hin, dass eine bestimmte Kohorte durchweg für die höchsten Wachstumsbeobachtungen während der gesamten Saison verantwortlich war. Größere Zuwächse sowie kürzere Häutungsintervalle wurden unter denselben Versuchsbedingungen bei Individuen festgestellt, die vermutlich aus Wintereiern geschlüpft waren. Der Kohorteneffekt war in einigen Jahren der Zeitreihe stärker ausgeprägt als in anderen. Insbesondere im Jahr 2019, welches auch aus fischereilicher Sicht ein Ausnahmejahr darstellte, wurden keine Wachstumsunterschiede innerhalb der Saison beobachtet. Der enorme Garnelenbestand im Jahr 2018 sowie ein vergleichsweise warmes Frühjahr 2019 wurden als mögliche Gründe für das Ausbleiben des Kohorten Effekts im Jahr 2019 erkannt. Die Auswirkungen auf die Wachstumsberechnungen wurden hervorgehoben, und der Schutz speziell der gut wachsenden Kohorte wurde als mögliches Managementziel genannt.

Neben dem Wachstumsinkrement stellt das Häutungsintervall (HI) den zweiten wichtigen Faktor zu Berechnung einer Wachstumsrate bei *C. crangon* dar. Da die Bestimmung des Intervalls zeitraubende und aufwändige Laborexperimente erfordert und die Ergebnisse stark abhängig von den jeweiligen Haltungsbedingungen sind, ist der Bedarf an einer Methode zur In-situ-Bestimmung des HIs aus Feldproben groß. In Manuskript 4 wurde eine ebensolche entwickelt. Auf der Grundlage des Prinzips der Instantaneous Growth Rate (IGR) wurde das HI für eine bestimmte Längenklasse anhand der sich kürzlich gehäuteten Tiere innerhalb einer Feldstichprobe berechnet werden. Um die frisch gehäuteten Tiere zu identifizieren, wurde ein Gerät entwickelt, das die Carapaxhärte mit einer aus der Werkstoffkunde bekannten Methode zur Härtebestimmung empirisch bestimmen konnte. Durch Validierung des Geräts mit im Labor beobachteten Häutungen konnten in gefrorenen Feldproben ein Sich frisch gehäuteter Anteil an Krabben innerhalb einer längenklasse ermittelt werden, und so ein Längenspezifisches HI berechnet werden. Dies stimmte zu großen Teilen mit bereits existierenden Beobachtungen zum HI überein, zeigte jedoch, dass HI gerade bei größeren Individuen von C. crangon kürzer ausfallen können als bisher angenommen.

2

General Introduction

Decapod crustacean species are the target of fisheries worldwide and form the basis of profitable aquaculture industries. While in the aquaculture sector primarily larger tropical shrimp species from both fresh- and saltwater habitats are bred (New, Michael, 1990; Boyd & Clay, 1998), commercial fisheries for naturally occurring shrimp focus primarily on saltwater species. In Europe the catch of a particular saltwater shrimp species, the common brown shrimp (*Crangon crangon*), represents a part of the shellfish industry that operates especially in the southern North Sea region. Due to an extended spawning period, a short life span and the lack of moult enduring hard structures, age determination in common brown shrimp is merely impossible (Boddeke, 1981; Oh & Hartnoll, 2004; Kilada & Driscoll, 2017). Hence the most recent stock assessments, as well as potential benefit estimations of management measures, are solely based on alternative growth estimates of the species, rather than age compositions of the stock (Temming *et al.*, 2017; Günther *et al.*, 2021). Although the growth literature for common brown shrimp is abundant (Hufnagl & Temming, 2011b), and several factors influencing growth in the shrimp are already identified, variability in growth was very large in almost all conducted studies to date (Hufnagl & Temming, 2011b) and much of it left unexplained. Thus, the main objective of the present work was to further investigate the growth process of brown shrimp and achieve a quantitative understanding as a basis for future assessment and management.

Biology of the common brown shrimp

C. crangon is a widespread species that can be found in almost all intertidal and littoral habitats of the northeast Atlantic. It´s distribution ranges from the coast of Morocco over the Atlantic coasts of Portugal, Spain, and France up to the North and -Baltic Sea. Further populations are found in the Mediterranean-, the Black Sea and off the coast of Norway and Iceland

(Luttikhuizen *et al.*, 2008). The common brown shrimp reaches a maximum length of around 90 mm and with a commercial size staring at around 50 mm is considered to be one of the smallest shrimp species caught for human consumption. Although the maximum age in the species is considered to be around three years, due to extensive fishing few animals live beyond one year (Hufnagl *et al.*, 2010c). The common brown shrimp tolerates temperatures between -1.4 and 30 °C (Berghahn, 1983; Reiser *et al.*, 2014a; b) and salinities from 5 to 35‰ (Criales & Anger, 1986; Gelin *et al.*, 2001; Cieluch *et al.*, 2005).

Because of its tolerance to fluctuations in both temperature and salinity, it is optimally adapted to the ever-changing environmental conditions of the Southern North Sea, where most of the commercial fishing occurs (Schulte *et al.*, 2020, ICES 2021). Here the benthic species, both hiding from potential predators and lurking for trespassing food particles, is mostly found burrowed, in soft bottom substrates, but also on sandy or stony ground close to the North Sea shore (Beyst *et al.*, 2002). Although *C.crangon* can be found and caught almost everywhere in the southern North Sea, the different life stages prefer different water depths and habitats, which leads to a seasonal migration of the shrimp population between close shore mudflats with 0-2 meters water depth and further offshore habitats, with water depths ranging from 20-40 meters (Boddeke, 1976; Schulte *et al.*, 2020). During winter large parts of the shrimp population prefer to dwell in deeper slightly warmer waters. With onset of spring and steadily rising water temperatures adults start to migrate closer to shore were water temperatures increase faster. Parallel, incoming recruit waves of freshly settled juveniles (5-20 mm length) arrive at shore in late May and the beginning of June (Temming & Damm, 2002). It is assumed that an active tidal stream transport enables longer distance migration within a short timeframe (Hartsuyker, 1966; Kuipers & Dapper, 1984; Hufnagl *et al.*, 2014). While selective tidal stream transport (STST) is known to occur in adults of the common brown shrimp and early live stages of other crustaceans (López-Duarte & Tankersley, 2009; Criales *et al.*, 2011; Hufnagl *et al.*, 2014), STST in brown shrimp larvae have not been proven yet. When juvenile brown shrimp arrive on the mudflats, they benefit from an abundant food availability and warm water temperatures. The mudflats, thus considered nursery grounds for the common brown shrimp, stay the preferred habitat during summer (Cattrijsse *et al.*, 1997). While small individuals prefer these intertidal habitats, with increasing size and sexual maturity, individuals tend to seek deeper waters again. By the end of summer, and with decreasing water temperatures, only few individuals remain close to shore (Boddeke, 1976).

Besides migration, primarily a distinct reproductive behavior of the species leads to changing abundances during season and between habitats (Temming & Damm, 2002; Hünerlage *et al.*, 2019). The common brown shrimp spawns throughout the year, with winter month proving to be more productive compared to especially late summer month (Siegel *et al.*, 2008; Tulp *et al.*, 2016; Hünerlage *et al.*, 2019). While in March up to 80% of the mature females carry eggs, in September and October less than 5% do (Hünerlage *et al.*, 2019). In general one distinguishes two different types of eggs, which are formed depending on the respective season and water temperature (Boddeke, 1981). The two egg types differ in their nutritional content and size. Females in summer produce more, smaller eggs compared to fewer, larger eggs in winter (Pandian, 1967; Boddeke, 1981). The transition from summer to winter eggs, and vice versa, is thought to be smooth, so that in fall and spring both egg types are found simultaneously (Urzúa *et al.*, 2012; Hünerlage *et al.*, 2019). The larvae that emerge from the respective eggs are called summer egg or winter egg recruits. Juveniles (5-20mm), hatched in February and March from eggs that developed during winter and recruit into the adult stock in May-June are considered winter egg recruits (Temming & Damm, 2002). Those recruits occurring in subsequent months are likely to be hatched from April onwards, and are hence called summer egg recruits. The occurrence of individual recruitment waves strongly depends on a synchronized larval hatch and high survival rates of the early live stages, which in case of winter egg recruits can be triggered by a rise in water temperatures after winter an a match of the offspring with the spring plankton bloom (Temming & Damm, 2002; Saborowski & Hünerlage, 2022). Summer egg recruits, which due to higher water temperatures on the date of their hatch, need less time to reach the post larval stage, and no temperature increase synchronizes hatching, summer egg recruitment is believed to less distinctively. The arrival of new recruits, especially winter egg recruits, often leads to a sudden increase in density in near shore habitats. Within a few weeks, Between May and June, densities can increase from a few individuals per square meter to over 100, especially in the nursery grounds preferred by juveniles (Temming & Damm, 2002; Penning *et al.*, 2021).

The ecosystem role

Due to its omnipresence and its high densities, the common brown shrimp is part of the prey spectrum of a large number of other species found in the North Sea. In addition a variety of fish species, such as cod and whiting, but also flatfish, seabirds feed on common brown shrimp

(Berghahn, 1983; Walter & Becker, 1997; Lancaster & Frid, 2002). Besides being prey, *C.crangon* is also predator of various mainly benthic species. The preferred food seems to change several times during the ontogeny of the common brown shrimp. While the pelagic larvae are primarily dependent on micro plankton, post larval individuals prefer larger plankton such as foraminifera as well as copepods (Boddeke *et al.*, 1986). The diet of juvenile shrimp then also includes the larvae and juvenile stages of a wide variety of benthic organisms such as mussels, bristle worms, nematodes and other crustaceans (Pihl & Rosenberg, 1984a; b; del Norte Campos & Temming, 1994). Adults of common brown shrimp are known to feed opportunistically and ingest almost all food particles that are in the right size range. These include algae, juvenile mussels, bristle worms, but also larval and juvenile fish such as plaice (Van Der Veer & Bergman, 1987; Wennhage & Gibson, 1998; Van Der Veer *et al.*, 2000). Stomach fullness in common brown shrimp coincides with the maximum activity level of the species and peaks at night (del Norte Campos & Temming, 1994). Because of its wide prey range and mass abundance, the brown shrimp is considered a key species in one of the most productive ecosystems in the world, by providing energy transport to higher trophic levels. In addition to this immensely important function for the ecosystem, the species is also of serious economic importance, since humans, as the highest trophic level, also benefit from the species' energy transport.

Fishery and Management

With around 500 active vessels, the three mainly participating nations, Germany, the Netherlands and Denmark land between 20 and 40 thousand tons annually. Only in 2018 the fleet landed an all-time high of 42 thousand tons yielding around of 120 million euros (STECF, 2020). Catch figures in *C. crangon* vary largely between years, indicating strong fluctuations in the actual size of the stock (Tulp *et al.* 2017). To date no factors other than the fishery were identified, as drivers for the stock size. Fishing for the common brown shrimp in areas of the Southern North Sea has a long tradition. While before the industrialization, quantity and quality of the brown shrimp catches remained low due to fishing methods used at the time, by the middle of the 19th century a change from subsistence to commercial fishing began (Lotze, 2007). The partial motorization of shrimp trawlers began during the First World War. Where at first only a winch was operated by means of an internal combustion engine, the boats themselves soon ran on diesel power. Due to an enlargement of the engines, at the time of the Second World War, the use of one net, trawled behind the ship, was replaced by the use of two nets on each side of the vessel. Initially used primarily as fertilizer in agriculture, or as an additive in animal food production, with guaranteed cooling chains and transport inland, common brown shrimp became a delicacy like many other marine products. After the gadoid outburst in the 1970s, heavy fishing pressure on the stocks of cod and whiting, led to a decline in the two main predators of common brown shrimp. After a longstanding opinion that C. crangon cannot be overfished, as natural mortality (M) was far higher than fishing mortality (F) (Tiews, 1970; Redant, 1980; Welleman & Daan, 2001). The role of these two predators was also taken over by the fishing industry through the further modernization of the fishing fleet. Today, the fishery is considered the primary driver of mortality in the shrimp stock, and steadily declining landings at slightly increasing effort levels suggest that fishing may even have an impact on recruitment (Respondek *et al.*, 2022).

Despite the fishery operating in a largely protected national park areas and the immense annual catches, the fishery was not a target of management measures until 2016. Here, following the advice of ICES in 2014 in the course of the certification process of the Marine Stewardship Council (MSC), a so-called self-management plan came into force. Prior to the self-management, only the maximum number of fishing vessels and a minimum mesh size of 16 mm were required by the EU (ICES 2015). From 2016 on, a stepwise mesh size increase of the codend to 26 mm was introduced to prevent growth overfishing. Further should a so called Harvest control rule (HCR) prevent recruitment overfishing by enforcing short-term reductions in effort at signs of falling stock sizes. The potential impacts and benefits of the management measures introduced, were estimated using a population-specific yield per recruit model (Temming *et al.*, 2017; Günther *et al.*, 2021). The model, which simulates a virtual shrimp population over the course of a season, is parameterized to reproduce the seasonal recruitment and catch pattern of the fishery based on the prevailing temperature conditions and the respective fishing effort in a mean year (Temming *et al.*, 2017).

Due to the lack of aging technics, an extended spawning period and the short lived nature of the species, cohort tracking or age based population models were found inapplicable for common brown shrimp. Thus, the recent modelling approaches were solely based on growth data, derived from a meta-analysis of growth studies on the species by Hufnagl and Temming 2011 b (Temming *et al.*, 2017). The calculations and outputs are hence strongly dependent on the growth input given. Although the model is able to predict the catch of the fishery at a constant recruitment input, so far it has not been possible to parameterize the biological inputs such that the adult stock surviving the fishery produces a number of eggs that will result in realistic catches in the following year (Günther *et al.*, 2021). The estimated overproduction of eggs can be the result of several factors, of which one is the growth parameterisation for the larger individuals is set to high. The variability in growth of shrimp within and between different studies, as well as some uncertainties concerning not yet investigated factors and their influence on growth, highlights the necessity of further investigations into growth of *C. crangon*.

Growth

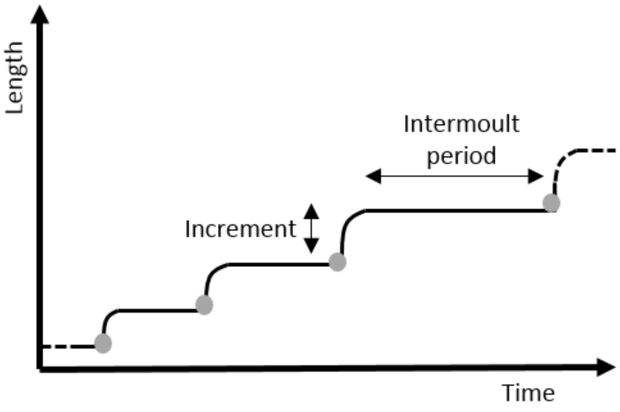

Figure 1: *Schematic figure of the interaction between moulting interval and growth increment*

As with all other crustacean species, the growth of the common shrimp is a gradual, rather than a continuous process. Two alternating mechanisms, the moult increment-, the actual increase in size immediately after moult as well as the moulting interval-, the period between two successive moults in which the individual hardly increases in size, determine the rate of length increase over time (Fig. 1). During advances in growth research of the species, the two growth defining processes, have received different levels of attention. And although both are needed to calculate a growth rate, the moult increment seems to be far better understood, and supported by a way larger data base than the intermoult period (Hufnagl & Temming, 2011b). This is primarily due to the efforts required for the observation of the respective processes. The growth increment is commonly derived from two lengths measurements before and after moulting. Hence it is comparably easy to obtain, especially in small fast moulting species like the common brown shrimp. To record individual moult intervals, animals must be observed for at least two consecutive moults. The dilemma that usually arises here is that during this time the animal is significantly influenced by the housing conditions in the laboratory, which makes the subsequent evaluation of the results error prone. All growth studies conducted on *C.*

crangon up to 2011 were combined in a meta-study, revealing 5 factors influencing the growth of the common brown shrimp (Hufnagl & Temming, 2011a; b). These are listed as follows:

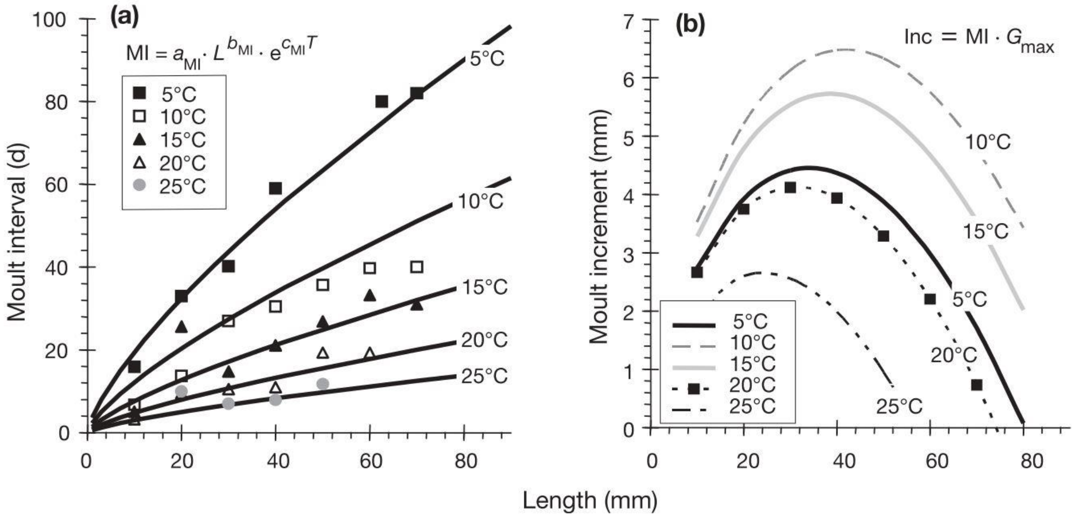

***Figure 2**: Moulting interval and moult increment at different temperatures over size (figure 4. In Hufnagl & Temming, 2011b). The graph is based on the results of all growth studies on C. crangon up to 2011 combined in the Meta analysis By Hufnagl and Temming (2001b).*

Size: Maximum moult increments were found in animals between 35 and 40 mm in length. Larger individuals showed significantly lower MI´s (Fig. 2 b). This is due to the difference in respiration and consumption. While the respiration depends on the weight of the animals, the consumption is mainly influenced by the surface area such as the intestinal surface (von Bertalanffy, 1934; Hufnagl and Temming, 2011a, b). Furthermore, the energy required for moulting is higher in larger shrimp, which leads to the animals showing longer intermoult periods and thus smaller growth rates. Individuals smaller than 35 mm show significantly shorter moulting intervals, and with increments that are only slightly smaller than those of the animals between 35-40 mm, greater growth rates can be achieved at high temperature, especially due to the shorter moulting intervals. In summary, the growth of C. crangon decreases with increasing size.

Temperature: The authors identified temperature as probably the most decisive, and also the best studied factor. Temperature had a positive impact on growth, especially for smaller shrimp under ad libitum feeding conditions. Higher temperatures resulted in an increased food intake, which according to Hufnagl and Temming (2011a) seemed to compensate for the increased respiration with temperature. As a result, the animals grew better at higher temperatures. At a temperature of 25 °C - earlier for larger individuals - growth rates begin to decline (Fig 2). An investigation of the preferred temperature of *C. crangon* revealed that it is strongly dependent on the observed animal size. Thus, the preferred temperature varies from 16°C to 20°C for adults to 20-25°C for small juvenile individuals (Hufnagl & Temming, 2011a; Madeira *et al.*, 2012; Reiser *et al.*, 2014b; a). A critical thermal maximum of 33.8°C for determined by Madeira et al. (2012) is likely to fit only for juveniles, as other authors experienced high mortalities in adults at lower temperatures (Freitas *et al.*, 2010; Reiser *et al.*, 2014b).

Sex: Significant differences in growth between males and females were found. Females tend to show 15-20% better growth than males (Sharawy *et al.*, 2019). However these effects become apparent only at a certain size of around 35 mm. This leads to the fact that from a certain size, the sex ratio of catches is increasingly dominated by females (Hufnagl *et al.*, 2010a). The fishery, which primarily targets animals larger than 50 mm, therefore catches significantly more females than males.

Cohort: In growth experiments differences in the growth potential of two groups of animals could be observed, which could only be attributed to the seasonal origin of the respective groups (Hufnagl & Temming, 2011a). Very low growth rates were observed early in the year (May) in animals of about 30 mm length. Several weeks later in July, animals of the same length showed growth rates higher than previously observed by any other author. The authors hypothesized that this was due to the feeding history and age of the individuals included in the two groups. While the individuals caught at 30 mm length in May were presumably overwintered summer egg recruits of the previous year which must have experienced periods of poor food availability in winter (Hufnagl *et al.*, 2010b). In contrast July individuals were likely to be winter egg recruits hatched in early spring. In further experiments from 2018 the comparison of growth in animals from August and September of the same size (30-40 mm), revealed that September individuals, presumably summer egg recruits, showed nearly no growth, while the August individuals, presumably winter egg recruits, under the same conditions showed growth rates, which even exceeded ones reported by other authors (Heimann 2018). Except for these two studies, there is no systemic evidence of a cohort effect. To date, it is unclear which factors dominate the occurrence of a cohort effect and which factors are ultimately responsible for the differences in growth between the two cohorts. The occurrence of the different recruitment waves has been studied by different authors, but there is a consensus that the winter/early season temperature is crucial for the synchrony and the strength of the winter egg recruitment.

Food availability: An adequate and varied food supply has been proven to be beneficial for growth of C. crangon compared than a one-sided scarce food supply (Oh & Hartnoll 2000; Uhlig 2002, Hufnagl & Temming, 2011a). It was further found that offering live plankton or nauplii of Artemia salina resulted in the higher growth rates than feeding frozen mussel or fish (Meixner, 1969; Uhlig, 2002a; Hufnagl & Temming, 2011a). Food supply may vary with season and it was demonstrated that food shortages, even in a highly productive ecosystem such as the southern North Sea can indeed occur, and also in a generalist feeding type species like *C. crangon*, (Hufnagl *et al.*, 2010b). According to Hufnagl et al. (2010), periods of starvation are found primarily in winter, although, there are indications that a certain percentage of the common brown shrimp stock shows signs of starvation even in periods when most animals are in good nutritional condition (Perger & Temming, 2012). By reducing its metabolism to a minimum *C. crangon* is believed to cope well even with longer periods of poor food availability. However it was demonstrated, that starvation severely influences growth, by both decreasing the moult increment and by delaying upcoming moults (Oh & Hartnoll, 2000; Uhlig, 2002; Hufnagl *et al.*, 2010). While a delay in moulting under starvation is known to occur due to the lack of energy necessary for moulting, and could be observed primarily in larval stages of several crustaceans, the reduction in dry weight during starvation was found to significantly reduce the subsequent moulting increment (Perger & Temming, 2012). To date, it is unclear to what extent prolonged periods of starvation, as they occur during winter, followed by a period of adequate food availability, affect the growth and mortality of individuals. Also the reduction in growth with food shortage in the population remains unquantified. Although brown shrimp are thought to be well equipped to withstand prolonged periods of starvation, the impact on stock productivity over the course of the season remains unclear.

Laboratory effects: The sixth factor that should not play a role as a direct influence in the natural environment of the common brown shrimp, but did play a role in the work summarized in the meta study, is the circumstances associated with keeping the shrimp in the laboratory (Hufnagl & Temming, 2011b). Studies on potential stressors as well as research on the use of the species in aquaculture (Lagadére, 1982; Dubber *et al.*, 2004; Stoner, 2012), identified several factors during capture and transport as well as while handling during husbandry that could lead to stress-induced behavioral changes in *C. crangon* and associated influences on growth. Besides diseases, which could be transferred to conspecifics via high density keeping in the lab, cannibalism, as well as water quality and pollutant load are mentioned here (Dauvin, 2008; Delbare *et al.*, 2015; Segelken-Voigt *et al.*, 2018). Furthermore, the common brown shrimp was found to be sensitive to noise, which led to a reduction in reproduction and growth, as well as increased aggressiveness and cannibalism (Lagadére, 1982). Haul duration, high temperatures, air exposure and the sieving processes on board of commercial vessels were found to increase mortality after capture (Lancaster & Frid, 2002; Gamito & Cabral, 2003; Temming *et al.*, 2022) and should thus also be avoided when collecting *C. crangon* for a laboratory environment.

Proxies for growth

Growth experiments are the most common way in order to investigate growth in shrimp. Although they enable the highly desired observation of actual growth rates in single individuals, the large variability in growth within the studies undertaken to date shows that factors exist that cannot be satisfactorily controlled even in laboratory experiments. In the review of growth studies, the brown shrimp was generally cited as difficult to maintain, and observations of poor growth in the lab are common (Hufnagl & Temming, 2011b). This was mostly attributed to the stress level of individuals kept in the laboratory, but also to sub optimal feeding regimes (Uhlig, 2002b). The use of alterative growth proxies based on field data can circumvent the uncertainties associated with laboratory experiments. Among others the RNA and DNA ratio, a proxy for the protein synthesis and thus for growth, was tested to explain part of the variability in growth of *C. crangon*, but turned out to be unsuitable as a direct growth proxy (Hufnagl & Temming, 2018). Perger and Temming (2012) found that the size of the moult increment is a function of pre moult dry weight at a given length. Refined in 2019, the method allows for a sex specific growth increment prediction for a group of individuals (length class), based on their dry weight condition (Sharawy *et al.* 2019).

The influence of the moulting interval

While the moult increment can be predicted based on the dry weight in field samples, for calculating growth rates, the method of Perger and Temming (2012) still requires the determination of a moult interval from laboratory experiments. As for the instantaneous growth rate (IGR) method (Quetin, L. B., Ross, R. M., & Clarke, 1994), the moult interval is calculated on the basis of a fraction of animals that have been moulted after a short stay in the laboratory in relation to the total number of animals stocked. To date, no method exists that allows the determination of the moulting interval on the basis of field samples. Although the properties of the exoskeleton changes-, and the dry weight at a given length follows a periodic pattern during the moulting cycle (Passano & Waterman, 1960; Perger & Temming, 2012), no study tried to use these information to predict recently occurred moults in frozen field samples.

Objectives of the study

Based on the previously described factors influencing growth, the present work was designed to further narrow down the variability in the growth of *C. crangon* and to hereby improve the growth input in the population model (Temming *et al.*, 2017). Therefore I addressed four factors as potentially influencing common brown shrimp growth, mainly using the outcome of a period of extensive growth experiments conducted between 2018 and 2021. Of the five factors that influence growth presented by Hufnagl and Temming (2011 a), I investigated two of them more closely, as these required further investigations. Besides starvation with subsequent re-feeding which can be attributed to “food availability” and cohort, I investigated the effects of population density on growth, and developed a method which allows for in situ moulting interval determination in C. crangon. Hence the research objectives can be summarized in four groups:

Chapter 3: As common brown shrimp frequently experience longer periods of starvation, how does starvation with subsequent re-feeding influence growth and mortality? Answering this question required:

- Laboratory starvation experiments with a subsequent re-feeding phase.
- The determination of body own energy reserves after re-feeding via analysing dry weight of both the individual and its exuvia after moult.
- Quantifying the occurrence of starvation in the field and evaluating the potential consequences for the stock productivity, using an incomplete series of dry weight condition data.

Chapter 4: Does increasing field density negatively influence growth in common brown shrimp in otherwise largely productive summer month? Addressing this objective included:

- Assessing shrimp condition and growth caught at largely different in field densities, by using three different approaches (growth experiments, dry weight condition analysis and size composition analysis in commercial and survey catches).
- Comparing natural fluctuations of density, to a potential density increases due to a management measure such as mesh size increase/ effort reduction

Chapter 5: How does the seasonal origin of shrimp influence their growth potential (Cohort effect)? This question was answered by:

- Assessing growth potential of same sized shrimp, caught in different seasons, during elaborate growth experiments.
- Investigating changes in shrimp condition during season, and calculate potential growth increments based on the results.

Chapter 6: Can moulting interval in C. crangon be calculated based on frozen field samples? Answering this question required:

- The development of a new method, in order to empirically determine exoskeleton hardness, for the use in determining recently occurred moults in field caught individuals.
- The calibration of the method, via elaborate laboratory experiments in which the moulting of individuals of the common brown shrimp was documented to the minute
- The use of the method in frozen field samples from different seasons

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3

Manuscript 1: Effects of starvation and subsequent re-feeding on moulting interval, growth increment and mortality in common brown shrimp (*Crangon crangon*)

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Growth, *C.crangon*, Starvation, Condition, negative growth

Abstract

Specimen of the common brown shrimp, *C. crangon*, (size range 21-40 mm) were deprived of food (0-16 days) and subsequently re-fed under controlled conditions (12h/12h light regime and 17°C water temperature), to investigate the effects of starvation with subsequent re-feeding on dry mass accumulation during intermoult period, mortality and growth. Mortality was negatively affected by starvation, and re-feeding was identified as particularly critical in animal survival, as over 90% of the observed mortality occurred after the onset of re-feeding. Starvation increased the moulting interval and decreased increments after moult, resulting in a shrinkage of up to 8% within 21 days. A correlation between weight loss during starvation and decrease in length was found. The comparison of dry weight conditions of starving animals and field samples suggested that shrinkage must be a more widespread phenomenon in *C. crangon* than previously assumed. Based on the observations, shrinkage could be identified as a physiological necessity after starvation. Accordingly, it could also occur in other crustacean species, in the cases were an individual’s dry mass is reduced due to starvation.

1. Introduction

Effectively dealing with food shortages is essential for the persistence of fast-growing and short-lived organisms. In the crustacean kingdom, a wide variety of adaptive mechanisms to food shortages have evolved, which in various ways enable animals to cope with even prolonged periods of poor food availability. While some species enter an inactive state, where locomotory, ventilatory and respiratory rates are reduced to a minimum (Hervant *et al.*, 1997), others respond to food scarcity by suspending their moult or even with shrinkage (Cockcroft & Goosen, 1995; Marinovic & Mangel, 1999). While a delay in moulting under starvation is known to occur due to the lack of energy reserves necessary, and could be observed in, primarily larval stages of several crustaceans, shrinkage and the physiological processes behind it are far less well understood. It remains unclear if a low physiological condition (e.g. low dry weight) that was obtained during the intermoult period as a consequence of starvation is correlated to negative growth when energy for moulting processes becomes available.

The common brown shrimp (*Crangon crangon*) is a key species linking trophic levels in the North Sea ecosystem (Evans, 1984; Cattrijsse *et al.*, 1997, ICES 2022) and with annual landings generally above thirty thousand tons in the shrimp fishery (ICES 2022) the economically most important crustacean species in the southern North Sea. Although the shrimp fishery receives little political attention due to the absence of an EU- controlled management, the species has long been a focus of scientific interest due to the extensive fishery in an otherwise largely protected area (Wadden Sea National Park). Since 2016, the shrimp fishery has introduced a self-management system aiming at the certification of the marine stewardship council (MSC). The self-management involves a stepwise increase of cod end mesh sizes based on the diagnosis of growth overfishing (Hufnagl & Temming, 2015). Potential benefits of the mesh size increase were estimated with a population specific yield per recruit simulation (Y/R) model (Temming *et al.* 2017; Günther *et al.* 2021) using growth rate estimates of Hufnagl and Temming (2011b). These growth rates were deduced from a meta-analysis involving mainly laboratory studies. Since all subsequent conclusions from the model simulations are highly dependent of the actual growth rates used, further research into the nature of the growth process in *C. crangon* is essential.

Growth rates in *C. crangon*, as with all other crustacean species, can be quantified by using the growth increment (GI), defining the increase in length after moult, and the intermoult period (IMP), which determines the frequency in which one moult would follow another. While GI is of interest for growth related questions only, moult regularity is also of importance for various biological processes in the common brown shrimp, e.g., reproduction, regeneration and disease susceptibility and control (Juanes & Smith, 1995; Oh & Hartnoll, 2004; Segelken-Voigt *et al.*, 2018). Extensive research on growth of the species in the last decades revealed that growth is influenced by several factors. In addition to temperature, sex and size of the animals, the availability and quality of food is shown to mainly have an influence on the growth of *C. crangon* (Hufnagl & Temming, 2011a; b). In *C. crangon*, the quantity of dry matter accumulated during IMP was positively correlated with the increase in length after moult (Perger & Temming, 2012). Conversely, starvation, even over a short period of time, results in a decrease of dry weight condition and also in GI (Oh & Hartnoll, 2000; Uhlig, 2002; Hufnagl *et al.*, 2010). However, as in other crustaceans in which negative growth was observed, it has not been shown if a decrease of dry weight, as a consequence of starvation, is consistently correlated to negative growth in length (as vice versa demonstrated for positive growth), which would require particular attention in Y/R models.

Surprisingly it was demonstrated that food shortages, even in a highly productive ecosystem such as the southern North Sea can indeed occur, even in a generalist feeding type species like *C. crangon*, (Hufnagl *et al.*, 2010b). According to Hufnagl et al. (2010), periods of starvation are found primarily in winter, although, there are indications that a certain percentage of the common brown shrimp stock shows signs of starvation even in periods when most animals are in good nutritional condition (Perger & Temming, 2012). The ecological significance of these observations is unclear, as it is not known if this is rather a successful energy saving strategy of the species or a sign of an indication of insufficient food supply.

Thus, the present study addresses how variable periods of food scarcity affect both growth-defining processes, moult increment and intermoult period as well as mortality of *C. crangon*. Furthermore, the relationship between dry mass increase during in the IMP and length increment during the moult stage (Perger & Temming 2012) was tested to be also valid when a decrease of dry mass and subsequent moult would result in a negative increment. The analysis of field data from a time series is presented to provide information on how frequently starvation periods leading to negative growth occur under natural conditions.

2. Material and Methods

Sampling

C. crangon were sampled in Büsum (54°07'N, 08°51'E) during low tide, using a push net (Dimension= 144 x 23cm, mesh size 1.8 mm) at about one-meter water depth. Live animals were transported to the Institute for marine Ecosystem- and Fisheries Science, Hamburg in aerated transport boxes filled with artificial sea water. Animals caught for dry weight condition analysis were frozen immediately after catch and then stored at -20°C until further processing.

Experimental setup

Two different experiments were designed to investigate growth during food deprivation, in both animals that were maintained individually and in individuals that were kept together in large numbers in a tank at high densities. Due to growth results and the respective availability, animals that were observed individually ranged between 20-40 mm in length, whereas the animals examined in the group had a length of 20-50 mm.

Individual observations

A total of 381 Specimen were maintained over several weeks, during two different experimental runs in July 2008-, June, July, August and September 2017. After an acclimatization period of one day, the individuals were kept separately in small tanks (10x10x20 cm). Water temperature during the experiment was 17 ± 2°C and differed from the infield water temperature by a maximum of 6.3 degrees (12/12, day/night cycle). Animals were fed ad libitum with fresh nauplii of *Artemia salina* and small pieces of *Clupea harengus* and *Mysidacea spec*. until their first (initial) moult. After the first moult, which was intended to put all individuals on the same basic condition, they were carefully transferred into 250ml Kautex vessels. Fine mesh (150µm) attached to two sides of the bottle should allow a continuous flow of oxygen-rich water, preventing the possible penetration of any food particles. With the transfer of the animals, a respective starvation period was induced. A control group was treated similarly but was fed continuously (ad libitum).

After the respective starvation period, animals were re-fed the same diet as before the starvation, by adding the food particles carefully to the Kautex-bottles. Daily removal of leftover food and feces, as well as regular addition of fresh food particles, should have allowed optimal regeneration conditions after starvation. Following the method of Perger & Temming, 2012, shortly after the second moult (up to 12 hours – when shrimp still had a soft exoskeleton mostly in the morning after moulting at night), the exuvia was removed from the tank, rinsed in purified water and frozen at -20°C. The freshly moulted individuals were immediately frozen at -40°C until further processing.

Group experiments

Since a food shortage in the field affects the whole population and possible interactions between individuals can occur, in a second experiment around 1000 individuals were kept in a group over a period of 14 days. They were maintained in a tank with 120x120x40 cm dimension, which was connected to a closed recirculation system. No food was added to the tank during the two-week trial. Directly at the beginning of the experiment as well as after 5, 8, 11 and 14 days, a group of at least 10 individuals from each length class were sampled and frozen at -40°C until further processing.

Length, weight, dry weight and sex

Length was measured as total length (TL1) from the tip of the uropodes to the tip of the scaphocerite, both at the beginning of the experiment and after each moult (TL2, TL3). Measurements were taken manually first. To minimize stress during the measurement process, a lateral picture, taken shortly after each moult, was later used, to measure length more precisely using the Image editing and processing freeware, Image J. Wet weight was also determined at the beginning (W1) of the experiment and after each moult (W2, W3), by blotting the individuals dry on paper tissue (scale: Satorius ± 0.001g). Dry weight (DW) and exuvia dry weight (EDW) after the 2nd moult were determined after 24 h freeze drying (scale: Satorius ± 0.0001g). Sex was determined from the endopodites of the first and second pair of pleopods and by the presence or absence of an appendix masculina using a binocular. To minimize handling stress, sex was not determined prior to the experiment, but after the end of the experiment, on thawed animals.

Field Samples

To relate our findings from laboratory starvation trials to the field situation, in-situ dry weight condition data from a number of years between 2006 and 2020 was compared with pre-moult dry weight conditions observed in the starvation trials. The comparison of existing literature with the results of the present study should provide information on the number of starving/shrinking animals in the field. Except for a few samples taken in Meldorf (54°05'N, 8°56'E), the samples for the dry weight analyses were caught at the same sampling site and using the same method as for animals caught for growth experiments. After a short storage at -20°C the animals were thawed and blotted dry on tissue paper. Wet weight, Sex and dry weight were determined as described for the individuals from the growth trials. However total length was only measured from the tip of the uropodes to the tip of the scaphocerite, to the nearest millimeter using graph paper.

Length at dry weight, Condition indices and potential Increments after Sharawy *et al.* (2019)

Dry weight and pre-moult dry weight at respective length was described as:

$$DW = a \cdot {TL_3}^b$$

$$DW + EDW = a \cdot {TL_2}^b$$

With DW= dry weight, EDW= Exuvian dry weight, TL_2 = length after first- and TL_3 = length after second moult and with a = 0.00199/0.00394 and b = 2.92/2.72 (Sharawy *et al.* 2019) for females and males (f/m, respectively). Water content, pre-moult dry weight condition index (pre-moult DWCI) and dry weight condition index directly after moult (DWCI) were calculated as

$$Water\ content = \frac{W_3 + DW}{W_3} \cdot 100$$

$$DWCI = \frac{DW}{a \cdot {TL_3}^b}$$

$$premoult\ DWCI = \frac{DW + EDW}{a \cdot {TL_2}^b}$$

With W_3 = wet weight after second moult, DW= dry weight, EDW= exuvia dry weight, TL_2 = length after first- and TL_3 = length after second moult. Potential Increment was calculated for males and females separately using the method described in Perger and Temming (2012).

$$pot.Inc_2 = \frac{DW^b}{a} - TL_3$$

The constants a, and b were taken from a more previous study from Sharawy *et al.* (2019), in which constants a = 0.00199/0.00394 and b = 2.92/2.72 for females and males (f/m, respectively) were calculated for the two sexes individually and on a larger dataset, including varying temperatures.

3. Results

Intermoult periods

Intermoult periods (*IMP*) lasted from 8 (LC 20-30mm, control) to 36 days (LC 30-40; 16 days Starvation). With increasing duration of the starvation period (*S*), the following moult of the animals was significantly delayed (Linear Regression Model, r^2= 0.8, F= 898.8, p<0.001, Fig. 3-1). In Individuals, which were fed continuously, the average time between two moults was 11 days (Temperature in all trials 17°C). Although starvation period accounted for 83% of the variance in IMP, variation within one treatment-group was quite high. Single Individuals showed moulting intervals twice the mean of their treatment-group. At 17°C moult interval could be described as:

$$IMP(d) = 2.051 * S(d) + 11.085$$

No individual moulted prior to their respective re-feeding event. The time between the start of the re-feeding the subsequent moult was 11.8 ± 2.4 days and did increased only marginally over the starvation period (Linear Regression Model, r^2= 0.04, F= 9.57, p= 0.0022).

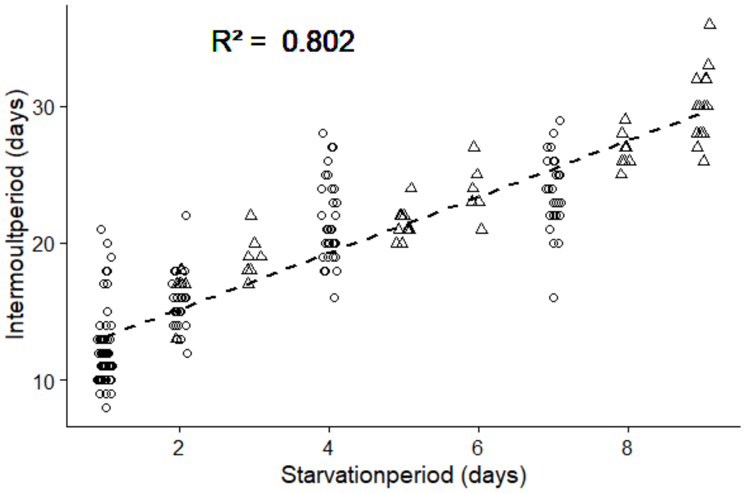

Figure 3-1*: IMP, the time between two consecutive moults (days), over starvation period during IMP (days). Triangles= study period 2008, circles= summer 2017, Individual's size was between 21 and 40 mm*

Increments

While the first increment was, with a few exceptions positive (Fig. 3-2 B) and did not significantly differ between treatment groups, the second increment was, depending on the treatment, partly negative and differed significantly the duration of the starvation period (ANCOVA,

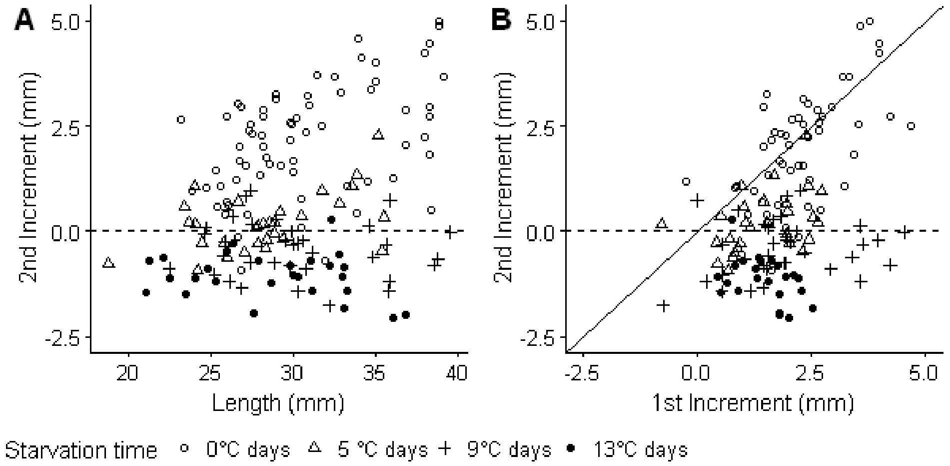

Figure 3-2*: A= Second increment (change in length after second moult and treatment) over length of the individuals. B= Increment 2 over Increment 1 (change in length after moult, before treatment). Circles = control, triangles= 5 days-, crosses= 9 days- and dots = 13 days starvation.*

F=10.72, p<0.001, Fig. 3-2 A). A food withdrawal over a period of 5 days lead to minimal or no increase in length. Longer starvation resulted in on average negative second increments.

Shrinkage occurred equally over the observed size spectrum. However, larger animals not only showed larger increments when fed (control), but also seemed to be more affected by food deprivation (Fig. 3-2 A). This resulted in a decrease of length of up to 8% (13 days starvation, LC 30-40 mm). When the two measured increments, both before and after starvation and re-feeding are compared, only the control group showed a similar growth performance after the first and second moult (illustrated by the diagonal line and circles in Fig 3-2 B). Individuals that were starved for more than five days were characterized by a positive first-, and a negative second increment (lower right panel of Fig. 3-2 B, dots and crosses).

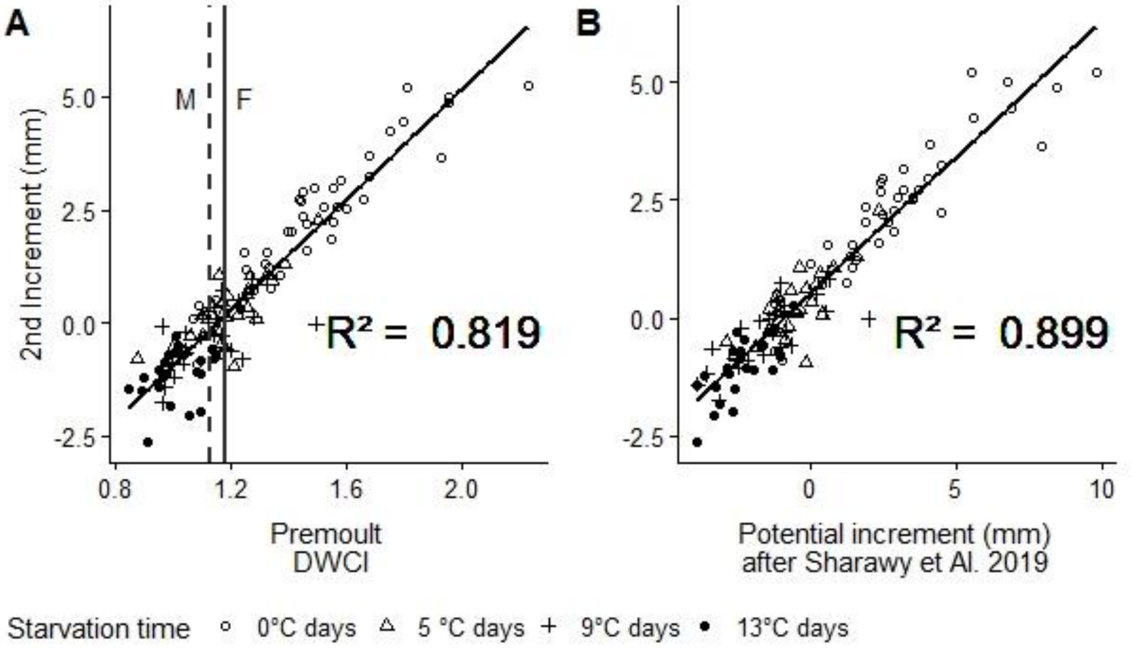

Figure 3-3*: A) Second increment over pre-moult DWCI with minimum condition after Sharawy et al. (2019) indicated by vertical lines for males (dashed) and females (continuous) respectively. B) Calculated potential increment after Perger and Temming (2012) over observed second increment in the starvation trials. Circles = control, triangles= 5 days-, crosses= 9 days- and dots = 13 days starvation.*

Based on the method of Perger and Temming (2012) with constants of Sharawy *et al.* (2019) a potential increment was calculated from the respective premoult dry weight condition for males and females separetely. The comparison of the actual observed increment and the calculated potential increment is shown in figure 3-3 B. There was a linerar relationship between observed and calculated increments that accounted for almost 90 percent of the variation, confirming that the method of calculating increments based on pre-moult DWCI is also valid for starving and shrinking individials. The combination of both, 2nd increment and moult interval, the growth rate, decreased significantly with the duration of starvation (ANCOVA, F=81.52, p<0.001). Negative growth rates of up to -0.12 mm per day (13 days starvation treatment) were repeatedly observed when individuals were starved for more than 5 days after their initial moult. Only a few individuals showed negative increments in combination with short intermoult periods. Starving animals were characterized by a long intermoult period and poor, partly negative growth increments, while animals that were fed continuously showed positive growth increments with short moult intervals. By combining growth increment and moult interval, the continuously fed animals can be quickly identified as winners (upper left half of the plot), whereas the starved and re-fed animals can be found as losers in the lower right half of the plot (Fig. 3-4).

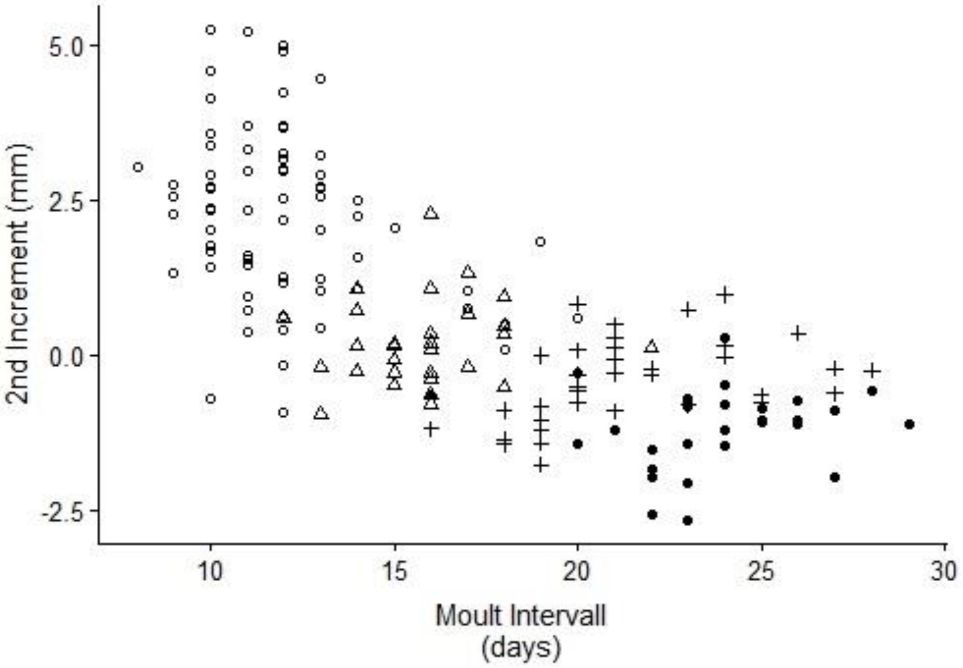

***Figure 3-4**: Second increment over moult interval. Circles = control, triangles= 5 days-, crosses= 9 days- and dots = 13 days starvation.*

Condition

Table 3-1: Dry weigh Condition thresholds at which the two observed length classes showed, no growth, 0.5 mm shrinkage, 1 mm shrinkage or 1.5 mm shrinkage.

	21-30 mm	31-40 mm
No growth	1.124	1.202
0.5 mm shrinkage	1.042	1.124
1 mm shrinkage	0.962	1.047
1.5 mm shrinkage	0.880	0.967

DWCI before moult (pre-moult DWCI) was with 1.5 ± 0.25 (Mean ± SD) significantly higher in the control group, compared to starved individuals (5 days starvation = 1.21 ± 0.1, 9 days = 1.13 ± 0.13, 13 days = 1.02 ± 0.08, ANCOVA, F=42.3, p<0.001). After moult however, starved animals showed only a slightly significant lower DWCI than the control group (ANCOVA, F 23.9, p=0.002). DWCI after moult was on a comparable level in the three starved treatment groups (Fig. 3-4). Based on the results of the starvation experiments, four dry weight condition thresholds were estimated at which no growth or 0.5 to 1.5 mm shrinkage was observed (Table 3-1). At a DWCI of 1.124, animals between 20 and 30 mm in length showed no growth, whereas animals in the 30-40 mm length class shrank an average of 0.5 mm. In general,

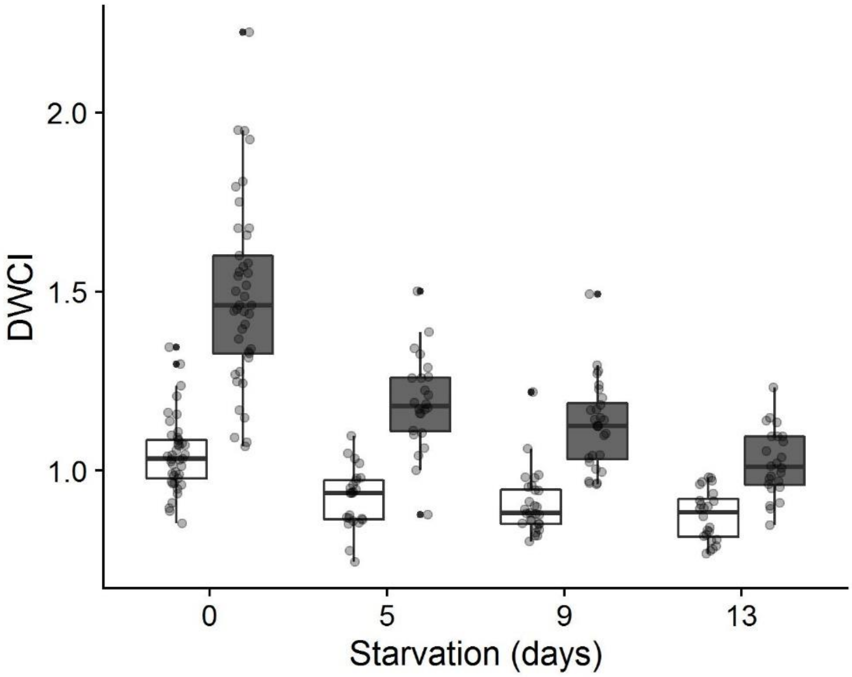

***Figure 3-5**: DWCI over duration of starvation period during IMP (days). White Boxplot= DWCI after moult, Grey Boxplots = pre-moult DWCI (length classes combined)*

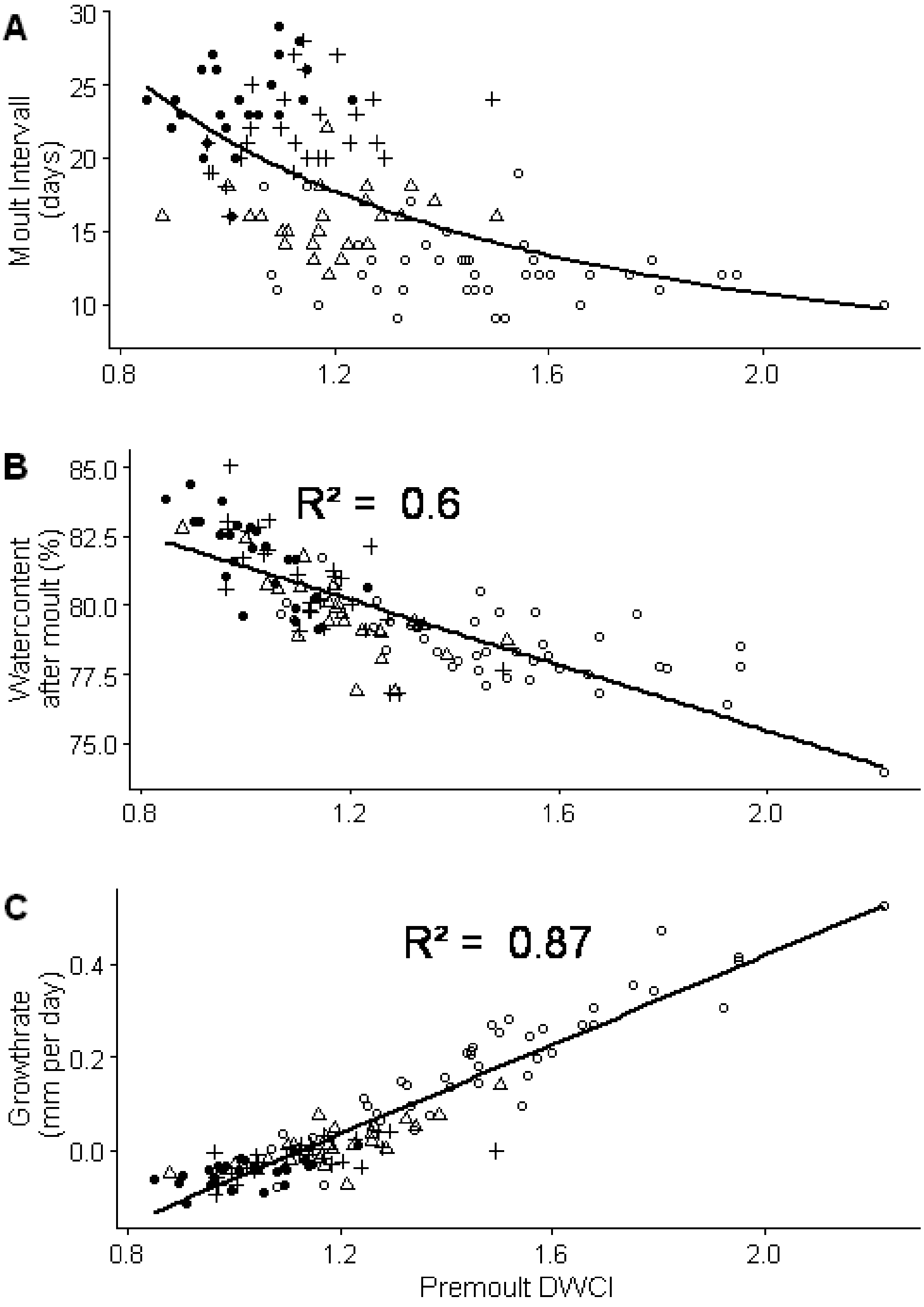

***Figure 3-6**: A= IMP over pre-moult DWCI, B= Water content pre-moult DWCI and C= Growth rate over pre-moult DWCI. Circles = control, triangles= 5 days-, crosses= 9 days- and dots = 13 days starvation.*

smaller animals needed less net dry mass at a given length, to maintain their size. With decreasing DWCI, the likelihood of the animal losing length after moulting increased. Watercontent, intermoult period, growth increment and growth rate were highly correlated to premoult-DWCI. Higher premoult DWCI led to significantly shorter IMP´s, larger GI´s resulting in higher growthrates (Linear Regression Model, r^2= 0.87, F= 776.8, p<0.001, Fig 3-4 C), and lower watercontents after moult (Linear Regression Model, r^2= 0.6, F= 174.8, p<0.001, Fig 3-4 B). Pre-moult DWCI explained nearly 70 percent of the variance in growth rate. Individuals that moulted with an pre-moult DWCI lower than 0.95, consistenly showed neagtive growth rates.

Starvation in a group

When held in groups, starvation reduced the condition of smaller individuals significantly (ANCOVA Length class 21-30 mm, F= 32.1, p<0.001; Length class 31-40, F= 26.7, p>0.001), while the condition of larger animals remained at a comparably high level throughout the entire trial period (ANCOVA Length class 41-50 mm, F=1.23, p=0.272). Compared to the animals that were kept individually, the group-starved individuals showed a DWCI below 1 only after 11 days and in the smallest observed length class. It should be noted, that the DWCI shown here, represents a condition that was not measured immediately after moult, and could originate from any point in the moulting cycle.

During the cumulative starvation trial, several incidences of cannibalism were documented. Here, larger individuals fed on either freshly moulted conspecifics of the same or smaller conspecifics. Furthermore, partly eaten animals could be observed.

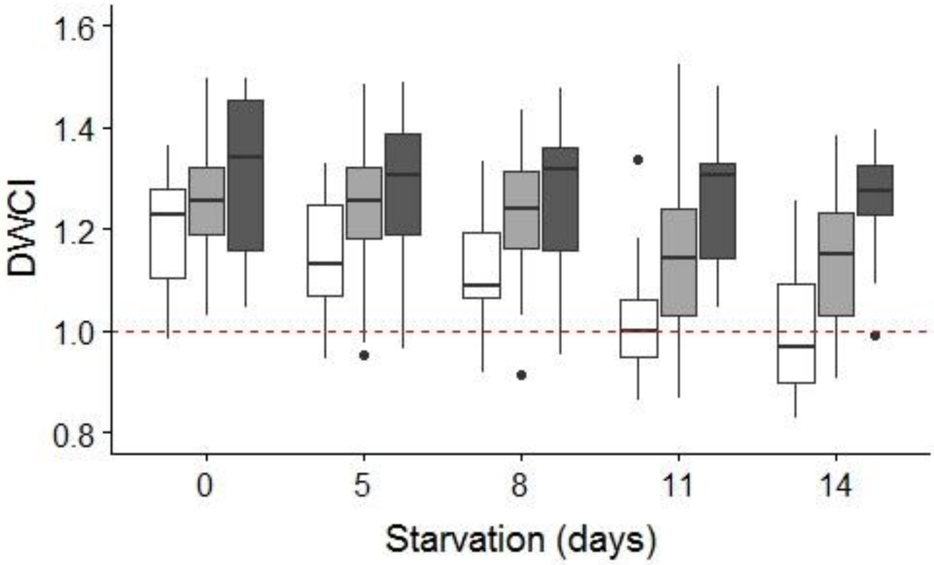

Figure 3-7*: Dry weight condition index for individuals starved in a group over their respective starvation period (days). White boxplots = length class 21-30 mm, light-grey boxplots = length class 31-40 mm, dark-grey boxplot = length class 41-50 mm*

Mortaliy

Mortality during the intermoult period was with 80.3 % at 13-, 72.2% at 9-, and 45.6% at 5 days starvation, markably higher in starved indiduals compared to the control group. Longer Starvation periods resulted in a rapidly, significantly increased mortality (Fig. 3-8) (Kaplan Meier p>0.0001).

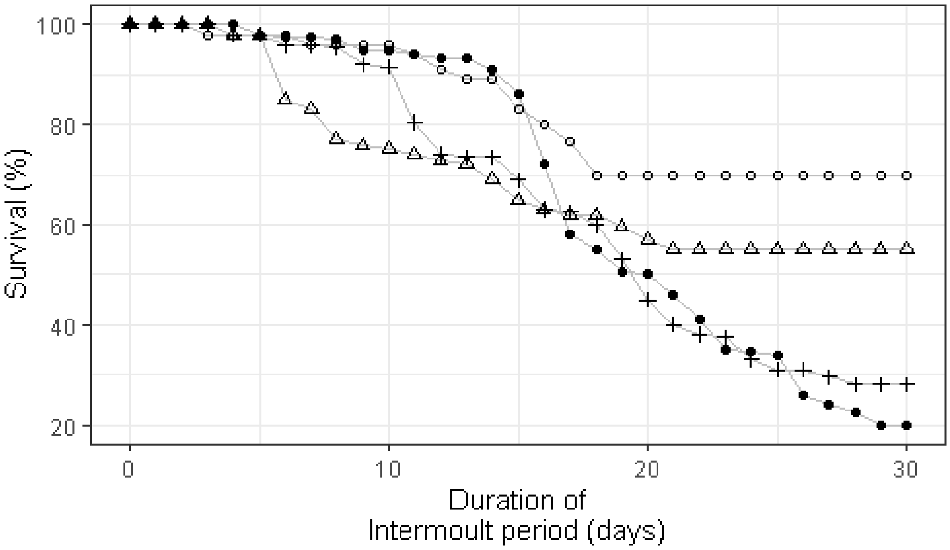

Figure 3-8*: Survival during IMP. Circles = control, triangles= 5 days-, crosses= 9 days- and dots = 13 days starvation. Dashed vertical lines indicate the respective feeding event for each treatment group.*

Only 8% of the total mortality in starved individuals occurred during the starvation period. The majority of the deaths were observed after the animals had been fed again (Fig. 3-8). In many of the dead individuals signs for the black spot disease were detected. Compared to the beginning of the experiment, these observations had increased drasticly. Four of the 237 deaths occurred while moulting. No observations were made on mortality of animals in experiment 2 (group housing). However, it should be noted that at the end of the experiment, clear traces of black spot desease were visible, the number of individuals in length classes 21-30 was greatly reduced, and more animals were observed eating conspecifics.

Condition Data from Field Samples

In order to search for starvation patterns in field samples and throughout the season, a condition value that indicates starvation and remained stable with fluctuating temperature was needed. In Perger and Temming (2012), a DWCI threshold was introduced, that described the lowest observed pre moult condition at which the animals were able to moult. This was later updated by Sharawy *et al.* (2019), and now also applicable down to temperatures of 9 °C. Individuals with a condition lower than 1.182 for females and 1.127 in males respectively, could hence be considered starving. According to the results of the present study, moulting at each of these conditions would have lead, to 0.17 mm growth in females, and 0.16 mm shrinkage in males. In other words, this condition would not have led to any visible change in length.

Table 3-2*: Number of Individuals and the proportion of starving and potentially shrinking individuals for the month may- November and the years 2006, 2007, 2010, 2019 and 2020*

2006	N=	710	126		465	395	504	202
	% Below min condition	57.0	17.5		49.9	62.8	50.4	80.2
	% 1mm shrinkage	21	3.9		24.1	37.2	20.2	37.2
2007	N=	213	376	336	241	90	205	250
	% Below min condition	30.5	59.3	35.1	5.8	24.4	30.4	70.4
	% 1mm shrinkage	6.6	23.4	8.9	0.4	5.6	9.2	24.8
2010	N=	803	1268	3457	400	450	1353	1763
	% Below min condition	26.5	9.6	45.2	25.6	20.7	17.7	57.6
	% 1mm shrinkage	4.7	1.3	20.2	5.8	0	0.3	30.3
2019	N=			198	103	107	40	194
	% Below min condition			55.1	27.2	28.0	32.5	65.5
	% 1mm shrinkage			22.7	4.9	5.6	5	23.2
2020	N=		220	200	40	380	64	97
	% Below min condition		80.5	46.5	7.5	35.0	45.3	55.7
	% 1 mm shrinkage		35	10.5	0	5	12.5	16.5

Since, the starvation experiments of the present study showed, that when starved for more than five days, moulting could also take place at a lower dry weight condition, another DWCI threshold was calculated in order to predict shrinkage in the field samples. With a DWCI of 1, the animals lost approx. 1 mm in length after moulting. Field samples that showed a DWCI of less than 1 at capture could therefore be considered “shrinker”.

A percentage of animals below each respective threshold was then calculated for the months of five illustrative years of the time series (Tab. 2). The proportion of "starving" animals according to Sharawy *et al.* (2019) varied between 5.8 % (August 2007) and 80.5 % (May 2020). In general, significantly lower proportions of starving animals occurred in the summer months (June-September). There was also a big difference between the individual years. While in 2010 generally better conditions were observed (highest share of starving individuals was 57.6 in November), in 2006 all months except June showed a share of starving individuals of more than 50 %. Compared to the calculated proportions of starving animals, the proportions of animals shrinking up to 1 mmm were significantly lower. These varied between 0% in September 2010 and August 2020, and 37.2 % in November 2006. The distribution of potential shrinking animals between the months and in the comparison of the different years was, as expected, analogous to the proportions of starving animals, although the percentages were significantly lower. Most of the potentially shrinking animals were observed in winter. Except for 2006, in the months of August, September and October, very few animals showed a condition that indicated negative increments in the following moult.

4. Discussion

Mortality

Mortality was highest in the 13 days starvation treatment and lowest in the control group. Continuously fed individuals showed with around 30 % a mortality that is comparable to other studies, performed over a similar period of time and at a comparable temperature (Hufnagl & Temming, 2011a). Various studies have shown that mortality of *C. crangon* in captivity can vary widely. Among the ways of conditioning and food availability, other factors, such as water temperature, stress-level and pollutant load of the water significantly influenced the shrimp's mortality (Lagadére, 1982; Dauvin, 2008; Stoner, 2012; Vermeersch *et al.* , 2018). Many of these factors, could be ruled out as the primary cause of deaths observed in the present study, since regular water analysis and the controlled experimental environment should have created optimal survival conditions.

Similar to findings of other authors, a significant increase in mortality was observed in starved individuals compared to continuously fed ones (Oh & Hartnoll, 2000). The difference between the present study and existing literature was a period of re-feeding after starvation. Here it was shown that it is precisely the phase after the starvation period that significantly increases mortality. Only 8% of deaths occurred during the starvation period. The other 92% were observed after the onset of re-feeding, suggesting that at the observed timespan, re-feeding seems to be more critical for the animal's survival than the period of food withdrawal. When starving, animals avoid energy consumption by reducing their metabolism and activity level to a minimum. This includes significantly reduced oxygen consumption- and increased ammonia excretion rates, indicating the usage of endogenous energy reserves (Regnault, 1980). When being fed again however, due to active digestive metabolism, significantly increased oxygen consumption rates, immediately after re-feeding, were observed in various other decapod crustaceans (Hervant *et al.*, 1997). This sudden increase in metabolisms after starvation, in which oxygen consumption rates exceed ones measured in normally fed individuals, could have led to the increased mortality by the onset of the re-feeding period. Furthermore, the delay of moult itself, and thus the interruption of all important processes associated with it, will have led to a higher mortality. The increased incidence of black spot disease suggests that the shedding of the shrimp's exoskeleton is required at shorter intervals. The ability to survive long periods of starvation without showing increased mortality, as reported by some authors (Regnault, 1980; Hufnagl *et al.*, 2010b), can be viewed critically in that the estimated mortality after re-feeding may be significantly higher than assumed without.

Increment

Moult increments ranged between -2.65 and 5.2 mm. In the animals that were starved and then fed again, moult increments were low, and on average negative for a starvation period exceeding 5 days. These observations are consistent with results from Oh and Hartnoll (2000), where shrimp fed only on every fifth day, showed significantly decreased growth increments compared to continuously fed animals. However no negative increments were observed in that study, suggesting that starvation over a period of 5 days could be compensated for by re-feeding. This, in turn, is in line with the observation from the present study, according to which individuals showed minimal or zero increments after five days of starvation (17°C), even though they had recently moulted and should therefore be in the worst condition within the moulting cycle (Perger & Temming, 2012). Hence feeding on every fifth day at a lower temperature and thus lower basic metabolism (Oh & Hartnoll, 2000) should have been sufficient for the animals to show positive growth rates.

Further, increments were highly correlated to pre-moult dry weight condition, equally if increments were positive, close to zero or negative, confirming that the relationship between pre-moult dry weight and increment introduced by Perger and Temming (2012) is also valid when the dry mass decreases during IMP. When starved, the animals fell far below the basic condition introduced by Perger and Temming. The approach of starting the starvation period immediately after a moult, chosen to put individuals in a comparable conditional state, forced the animals to compensate for the lack of food by reducing further endogenous energy. The additional dry matter lost, was not compensated by the subsequent re-feeding and until the next moult, which is demonstrated by an increased water content and decreased pre-moult DWCI in starved individuals. The DWCI after moult was only marginally smaller in starved animals compared to continuously fed ones, which indicates that even prolonged periods of starvation lead only to slight changes in the basic condition after moult as was suggested by Perger and Temming (2012). After moulting, the release of water absorbed during the starvation period, presumably leads to a reduction in length and thus to a renewed approach to the basic condition. Perger & Temming (2012) demonstrated, that the relationship between dry weight and length in normally feeding brown shrimp oscillates between a basic condition that is determined by an uptake of a specific amount of water during moult, and the highest amount of dry mass accumulated during the IMP, prior to an upcoming moult. The amount of the dry mass that was accumulated during an IMP was positively correlated to the water uptake, and thus the increase in volume and length in the following moult. The results of the present study suggest that conversely, a loss of dry mass during IMP, results in an ion concentration gradient during moult that is to the shrimp’s disadvantage, and leads to a decrease in cell size and length of the individual.

Intermoult period

Intermoult period, which in most of the previous growth studies on *C. crangon* was mainly described as a function of size and temperature, significantly differed with treatment, ranging from 12.2 ± 2.65 days in the control group to 30 ± 2.65 days at 16 days starvation. The prolongation of moulting intervals was shown to be typical response to poor feeding conditions (Oh & Hartnoll, 2002). Especially in larvae of several crustacean species, moult delay is seen as a reaction to a lack of energy, needed for the energy-intensive moulting process (Abrunhosa & Kittaka, 1997; Liddy *et al.*, 2003). The results of the present study show that adults of *C. crangon* also exhibit this behavior. The animals postponed their moult for approximately the same period of time as they were deprived of food. After the onset of re-feeding, a period of about 11 days passed, which was slightly more than the moulting interval of the control group and independent from the respective starvation period. This indicates that the animals used exactly this period to absorb the energy necessary for moulting. A moult then took place without compensating for the energy lost during starvation. Moult occurring at the expense of growth, suggests that the duration of the moult interval is determined by more important factors other than somatic growth. We would like to propose two hypotheses explaining these observations.

First, the exoskeleton is possibly critically affected by parasites such as black spot disease due to the prolonged IMP (Segelken-Voigt *et al.*, 2018), requiring the immediate shedding of the infected exoskeleton, as soon as the energy for moulting is obtained. Second, the decrease in length, likely being the consequence of water release, possibly re-establishes homeostasis in the shrimp cells. When normally feeding, protein uptake during the IMP may cause physiological stress due to low water content. Because of their exoskeleton and thus their fixed size, the shrimp lack the ability to absorb water, leading to a decreasing water content during IMP. At a certain point, high protein levels in the cells trigger moulting (Hartnoll, 2001). Water intake in the soft animals after moult restores homeostasis, which also leads to enlargement. After starvation events however, proteins are replaced by water and the water content of the cells may become too high, requiring water release and the decrease of their volume (and length). Accordingly, shrinkage after starvation periods maybe a physiological requirement in crustaceans in general.

Field condition

The fact that starvation can occur naturally, even in an omnivorous species living in a highly productive habitat such as the brown shrimp, has been demonstrated by Hufnagl *et al.* (2010). In this study animals were deprived of food in laboratory experiments and after a respective starvation period their dry weight condition was compared with dry weight conditions obtained from field samples. Especially in winter months, large parts of the shrimp population showed dry weight condition values that indicated prolonged starvation periods of up to 80 days. In a second study from Perger and Temming (2012), which primarily aimed at the development of a method for situ growth rate determination, a frequent appearance of starving individuals in field samples was reported. The use of a different criterion, i.e. falling below the basic condition after moulting, revealed that a share of the population seemed to be in a starving state even in summer month. Following the method Perger and Temming (2012) a more recent study of Sharawy et al. (2019) re-estimated the minimum condition at which individuals were barely able to moult, for the two sexes separately and reliable down to a temperature of 9 °C. Based on these calculations and on the results of the present study, two dry weight condition values were used in order to calculate the shares of starving and potentially shrinking individuals for five illustrative years of an incomplete set of field samples.

It was found that the shares of starving individuals, showing a condition, that should have allowed no growth if the individuals moulted on the day of their catch, varied greatly between different month and years of the time series. The predictions of potentially shrinking animals coincided with shares of starving individuals, calculated based Sharawy et al (2019). Although the proportion of animals that would have shrunk by 1 mm or more had they moulted on the day of capture was low, compared to the shares of starving individuals following the minimum condition of Sharawy *et al.* (2019), in some cases “shrinkers” accounted for almost 40 % of the total catch (September & November 2006).

Based on the observations of the present study, it can be assumed that these animals should indeed have shrunken when feeding again. Nevertheless, reports of negative increments are rare in the literature. One reason for this may be, that the frequently used instantaneous growth rate method (IGR) only takes animals that moult immediately after capture into account, which excludes animals that are starving and can only moult after being fed again. Furthermore, in many growth studies no growth is observed (Hufnagl & Temming, 2011b). It can be assumed that authors who found first increments close to zero, have almost certainly examined starving animals. If one now also considers the increased mortality in starving animals, it becomes clear why negative increments are so rarely observed.

In a previously unpublished series of growth experiments with *C. crangon* between 2019 and 2021, animals showed negative increments when they moulted 10-20 days after catch. This phenomenon was observed especially in the winter months, which suggests that the observations made in the starvation trials, combined with findings from the dry weight condition analyzes can certainly be transferred to animals caught in the field. Considering minor uncertainties, such as the significantly slower condition loss at lower temperatures (Hufnagl *et al*., 2010), and the lack of data on the effects of food deprivation on larger individuals, this could have implications, especially for the adult stock, in modelling approaches on the lifecycle and management of common brown shrimp (Temming *et al.* 2017; Günther *et al.* 2021). In winter month, larger adult shrimp dominate commercial catches, who are believed to mainly contribute to the recruitment in the following year (Temming & Damm, 2002; Temming *et al*. 2017). In case of starvation periods during this time of the year, a prolongation of the moulting interval, which plays a decisive role in the reproductive cycle of female brown shrimp, will potentially lead to a reduced egg production. This might influence the balance of the contribution of winter and summer egg recruits to the stock. Further does the share of starving individuals directly influence the mean growth rate of the population. Since based on the DWCI analysis, starving animals are present throughout the year, it can be assumed that the growth rates in current literature are probably overestimated. While in summer, slow grower will have little influence on the mean growth rate of the population, in winter when food is scarce, an over 50% of the population become slow growers, reduced mean growth should be considered. It remains unclear why in summer month animals with excellent condition and animals with a condition that indicates a long period of starvation can be observed at the same sampling site. Recent studies documenting the feeding behavior of animals in captivity show (Haghani, 2021), that even when fed ad libitum, many animals refuse to ingest the offered food. Stomach content analyses from field samples show that even in the field, there are always animals that show empty stomachs, although the majority of animals caught at the same location had ingested a variety of different food particles (Phil & Rosenberg, 1984).

If these observations are applied to crustaceans in general, and if regular moulting occurs at the expense of somatic growth, be concluded that in other species too, food deprivation would lead to moulting at a state of low dry mass condition, and thus to a decrease in length. Although In other decapod crustacean species, e.g. krill and lobsters, shrinkage is known to occur when insufficient amounts of food are available (Nicol *et al.*, 1992; B. Marinovic & M. Mangel, 1999; Dubber *et al.*, 2004), shrinkage could be a more widespread phenomenon than previously assumed.

Future studies should investigate the occurrence of starvation in the common brown shrimp population also in relation to factors such as population size and season. Furthermore, it should be investigated to what extent the occurrence of starving animals in field samples can be explained, although apparently the conditions for sufficient feed intake are given. The relationship between decrease in dry mass and a physiologically required moulting as well as shrinkage should also be tested in economically important crustacean species living in cold or temperate latitudes, as starvation events and negative length increments were already detected in several other crustacean species. Regarding commercial use and stock assessment, these findings must be considered.

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4

Manuscript 2: Density dependence in common brown shrimp (*Crangon crangon*), a three-way approach

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Abstract

In 2016 within the self-management plan of the common brown shrimp fishery, conserving measures were introduced, that may lead to a regional increase in density. Concerns regarding density-dependent effects, that would counteract the potential benefits expected from the conservation, prompted an investigation of these effects on common brown shrimp growth. In the present study, three approaches were chosen, to investigate the effect of density on 1. Actual growth observed in laboratory trials, 2. Dry weight condition in field samples and 3. The size composition of both commercial and survey catches. In summer, when the greatest effect of a conserving measure is expected, we found no differences in actual growth, dry weight or size composition between different densities within a short period of time, between different densities in different months of a year, and between densities in the same month of different years. In none of the three approaches, indications for a potential food limitation due to density was found. On the contrary, the best growth condition and best ratio of small to large shrimp were observed in samplings, performed at high densities, suggesting that shrimp growth in summer is bottom up controlled, rather than top down.

1. Introduction

Density dependence is a common theorem in population ecology and natural resource management. It occurs when fitness influencing processes, such as growth, reproduction or mortality change as a function of the number of individuals within a population. A distinction is made between processes, in which the population fitness is affected at high- and those at low densities. While in small stocks, mostly the less efficient reproduction leads to a reduction in stock fitness, in large stocks, increased intra-species competition for various resources, can lead to lower early life stage survival, slower growth and increased mortality (Lorenzen & Enberg, 2002). Aiming at maximum productivity, current management approaches try to avoid both scenarios. In single species fisheries management, the majority of the stocks are managed according to the principle of maximum sustainable yield (MSY). Measures such as fishing gear adjustments, but mainly a total allowable catch (TAC) or quota, is to ensure that the stocks in a size rage of maximum productivity. Examples of density-dependent growth in marine species at our latitudes include stock recruitment relationships of fish, such as North Atlantic flatfish species (Van Der Veer *et al.*, 2000), or food competition especially in the early life stages of different fish species (Lorenzen & Enberg, 2002). Also in crustacean species such as Norwegian lobster and common lobster signs for density-dependent effects on productivity were found (NELSON *et al.*, 1980; Chang, 1995; Da Silva Santana *et al.*, 2021).

One of the crucial crustacean species in the Southern North Sea ecosystem is the common brown shrimp (*C. crangon*). It is considered a key species in the North Sea Ecosystem, being both predator and prey for a variety of other species, and by linking the trophic levels in the ecosystem (Boddeke *et al.*, 1986; Van Der Veer & Bergman, 1987; Jönsson *et al.*, 1993). Besides its ecological value, *C. crangon* is the most valuable crustacean species caught for human consumption, with a 500 vessel strong fishery, landing between twenty- and forty thousand tons yearly (ICES ,2022). Despite a comparatively equal effort between different fishing years, there are large differences in the absolute amount of shrimp landed (ICES, 2022). A recent study found an annually changing productivity of the shrimp stock that varied by a factor of 5.6 between 1970 and 2015 (Tulp *et al.*, 2016). To date, factors that influence the productivity of the stock and hence the absolute number of shrimps in the population are little understood. However, a crucial point in productivity regulation is believed to be the availability of food for different life stages of the common brown shrimp and throughout the season (Hufnagl &

Temming, 2011a). Especially during winter, the common brown shrimp stock shows signs of food limitation (Hufnagl *et al.*, 2010b). Although stock sizes tend to be smaller in month between January and March, up to 80% of starving individuals indicate that food limitation can occur in *C. crangon*.

The steadily increasing influence of the fishery, and especially a certification of common brown shrimp fishery by the marine stewardship council (MSC) in 2016, required a management plan for the species. As management was not considered necessary by the EU, representatives of the three main countries involved in the shrimp fishery started developing a self-management system. To counteract potential growth overfishing in a previously largely unmanaged stock (Hufnagl & Temming, 2015), alternative concepts to the classic quota management were developed. Due to the short-lived nature of the species and the dynamic development of the population within a very short period, a harvest control rule was introduced, to quickly adjust effort in cases where previous landings per effort indicated a critical stock status. Further a gradual increase in cod-end mesh size was planned to prevent potential growth overfishing of the common brown shrimp (Hufnagl & Temming, 2015). Although potential benefits of the mesh size increase were estimated with a population-based simulation model for yield per recruit (Temming *et al.*, 2017; Santos *et al.*, 2018), increasing numbers of potentially surviving escapees, raised concerns of potential density dependent effects during summer. The increase in density due to the increase of the meshes could lead to greater intraspecific competition and thus to a reduction in growth or even increase mortality within the population. Various studies on intraspecific food limitation of decapod crustaceans in general (NELSON *et al.*, 1980; Pérez-Castañeda & Defeo, 2005; Wieland, 2005), observations on density dependent mortality in *C.crangon* in winter, as well as the fact that food limitation was known to occur in common brown shrimp in winter, suggest that the concerns might not be unjustified. In the Bristol Channel, adult stock of common brown shrimp showed a positive correlation between mortality and stock size, indicating that higher intraspecific concurrence over scare food availability leads to increased morality (Henderson *et al.*, 2006).

Next to observations on mortality, growth could serve as an indicator for population productivity (Moyano *et al.*, 2020). In the case of crustaceans, growth consists of two processes. In addition to the increment, the actual increase in length immediately after a moult, the moult interval provides information on how quickly the animal can increase in length. Especially early increments obtained closely after the catch of the individuals, as it is easy to obtain, and linkable to the field condition can be used for productivity classifications (Quetin, L. B., Ross, R. M., & Clarke, 1991). Another measure, that is also widely used for C. crangon is the mean dry weight at a given length, also referred to as dry weight condition (DWCI) (Hufnagl *et al.*, 2010b; Perger & Temming, 2012; Sharawy *et al.*, 2019). Based on the assumption that an animal of a given length can spend more energy on growth if net dry weight is higher compared to an animal with a lower dry weight value of the same length, a high DWCI indicates a better condition. If the individual condition of a sufficiently large number of animals is analyzed, conclusions can be drawn from the mean value for at least the part of the population located in the same region animal were sampled. Further can a potential increment be calculated on the basis of the DWCI, which again serves as a direct measure for growth and hence productivity (Perger & Temming, 2012; Sharawy *et al.*, 2019).

In the present study, three different approaches were chosen to investigate density dependent effects on growth and condition of the common brown shrimp. In the first approach, growth potential of individuals caught at different densities was analyzed during a set of growth experiments. Early post capture growth increments were compared between different densities, and to laboratory increments, observed after a period of ad libitum feeding, to detect potential food scarcity in the field. In a second approach the dry weight condition (DWCI) data, as an indicator for possible food limitation and nutritional fitness, was compared in field samples, caught in different years and at different densities. Based on DWCI, potential increment after Sharawy et al. (2019) was compared, to investigate changes in growth potential based on dry weight at different densities. Since the first two approaches examined regional limited data only, in approach three, commercial fishery- and survey data (DYFS) from several subareas of the German EEZ and the years 1997 to 2016 were analyzed regarding changes in length frequency distribution (LFD). If the absence of a large length class compared to sufficient amounts of the next smaller 10 mm length class indicates reduced growth, two successive length classes (51-60 and 61-70 mm) were compared in their relative numbers in the catch. Since the main effect of the density increase would be expected to occur in summer/fall (Temming *et al.*, 2017), specifically the summer months in the different data sets were examined for density-dependent effects.

2. Material and methods

Sampling

Animals, for both experimental runs and condition analyses, were sampled in Büsum, Germany (54°07 N, 08° 51 E) using a push net (dimensions: 1.2 m width x 20 cm height, 2 mm mesh size) in 0.3 to 1 meter water depth. The distance covered in the first haul of a respective sampling day was determined using GPS. Individuals for density determination and dry weight condition analyses were frozen immediately after catch and later stored at -40°C until they were further processed. Animals for the experimental trials were transported to the Institute for Marine Ecosystem- and Fishery Science, -Hamburg, in thirty-liter drums filled with artificial seawater, and under constant ventilation. A detailed description of the sampling methods, used for the years 2006 and 2007 of the time series can be found in Hufnagl *et al.* (2010). For the year 2010 a description is found in Sharawy *et al.* (2019).

Density

Prevailing density at the sampling site, was determined by dividing the total number of individuals caught in the first haul by the swept area, which in turn was calculated from the GPS measured haul-distance and the width of the push net. The total number of individuals was determined, by extrapolation of a weighed and counted subsample. For this purpose, the respective sample was carefully thawed, freed from by-catch and weighed (scale: Satorius ± 0.01g). Subsequently, a sub-sample was taken, its weight determined, and the animals contained therein counted. The mean individual weight from the sub-sample was then used to determine the absolute number of shrimps in the total catch. Density values for DYFS Data were obtained by calculating the total number of individuals caught within one haul and normalizing them to fishing hour and the net swept area. Therefore, the total catch weight was divided by the mean weight of an individual shrimp. Individual shrimp weight was calculated by using length measurements from a subsample (200 g) of each catch and a length weight relationship. Subsequently, the total numbers in the individual hauls from the German EEZ were grouped by year and station and normalized to covered area and fishing time.

Length, weight, dry weight and sex

In the present study different data sets were used, and based on different methods of length determination in each of the three approaches. Generally, length for all individuals was measured as total length from the tip of the uropodes to the tip of the scaphocerite. In the laboratory experiments (Approach 1) the length was measured digitally using Image editing and processing freeware, Image J. Length measurements in both the DWCI (Approach 2) dataset and the DYFS (Approach 3) dataset were taken to the lower millimeter, either using graph paper (DWCI dataset) or an electronic measuring tablet (DYFS). Dry weight (DW) was determined after 24 h freeze drying (scale: Satorius ± 0.0001g).

Sex was determined, either by the presence of eggs, or on the endopodite of the first and second pair of pleopods and by the presence or absence of an appendix masculina using a binocular. To minimize handling stress during laboratory trials, sex after the end of a respective experiment, on thawed animals.

Dry weight condition index and potential increment after Sharawy *et al.* (2019).

Since the constants from a length-dry weigh relationship using all data sources, only differed minimally from those of Sharawy *et al.* (2019) and for simplicity, the already published constants were used for any of the further calculations. Both DWCI and potential increment were calculated using the sex specific coefficients a= 0.00394/ 0.00199 and b= 2.72/2.92 for males and females respectively (m/f).

Dry weight condition index was calculated as:

$$DWCI = \frac{DW}{a \times TL^{b}}$$

With DW being individual dry weight and TL being total length. Potential increment was calculated as follows:

$$INC_{p} = TL \times \left(\frac{DW - EW}{a \times TL^{b}}\right)^{1/b} - TL$$

With DW being individual dry weight, EW being exuvian dry weight and TL being total length. Exuvian dry weight was calculated using an exuvian dry weight length relationship also provided in the 2019 publication of Sharawy *et al.* (2019):

$$EW = 0.000523 \times TL^{2.89}$$

The method of Sharawy *et al.* 2019 allows the calculation of a mean potential increment for a group of individuals, ideally a length class. For this purpose, only the potential increments of animals from the respective length class is calculated, which would have been positive based on their dry weight (individuals pot. Increment >0). For the final calculation of the potential increment for one length class the averaged potential increments of all individuals were multiplied by the factor two. Based on the assumption that the moult stages are homogeneously distributed within the population, the multiplication factor of 2 is necessary to compensate for animals that have recently moulted. For details, the study by Sharawy *et al.* (2019) can be consulted.

Approach 1 – Laboratory observations on growth

In the first approach, individuals caught at local different densities were brought to the Institute for Marine Ecosystem- and Fishery Science (IMF) in Hamburg and were then observed individually over the period of two successive moults in order to assess their growth rate. After an acclimatization period of about 24 hours, the animals were photographed (length measurements) and then kept in individual compartments connected to a recirculation system. The individuals were fed ad libitum with nauplii of *Artemia salina*, pieces of *Clupea harengus* and *Mysidascea spec*. two times a day while feces as well as left-over food was removed from the tanks. From the measured starting length (TL1) and the respective lengths after the first (TL2) and second moult (TL3), the moult increments were calculated. As the trials were carried out at different month of the year, the trial temperature had to be adjusted to be close to the respective field temperatures. A uniform temperature of 17°C was chosen for the summer months of all years for the sake of comparability. At no time did the experimental temperature deviate more than 6.5°C from the temperature in the field. The light regime was always 12h/12h (day/night).

To compare the individual's growth potential at different densities, two different proxies were obtained from the experimental trials. The recording of the 1st moult increment immediately after capture should have served as a proxy for growth that individuals would have shown in the field. The 2nd increment after an intermoult period at ad libitum feeding should have represented growth under "optimal" conditions. The comparison of both increments in the same individual should provide information on whether the ad libitum feeding improved nutritional condition and hence the growth rate of the animals. Improved growth increments after ad libitum feeding could have indicated a food shortage prior to the individuals catch in the field. In addition to moult increments as a proxy for growth, also mortality rates were documented throughout each trial. A trial was carried out over roughly the period of about 2 intermoult periods, usually 30 days, but depended on the respective experimental temperature and animal length. To compare mortalities in experimental trials with differing durations, mortality was determined after 30 days in all experiments. Accordingly, animals that survived to day 30 were considered survivors even if they died at a later point in time, and at experimental period exceeding 30 days. Growth potential was compared both at occasions in which density differed largely in a short time frame and between samplings of the same month but in different years. Since the expected benefits from a conserving measure such as a mesh size increase can primarily be explained by the sparing of the stock in summer (Günter *et al.* 2017), the selection of months that were sampled in 2018, 2019, 2020 and 2021, only concerned the summer.

Approach 2 – Dry weight condition data from field Samples

In the second approach field samples of *C. crangon* caught at different densities were analyzed with regard to differences in their dry weight condition. Therefore individuals, caught at the sampling site in Büsum, were packed in small groups, brought to the Institute for marine Ecosystem and Fisheries science in Hamburg on crushed ice, and were then frozen on -40°C until they were further analyzed. After thawing their length was measured to the nearest millimeter, wet weight, sex and dry weight after 24h freeze drying was determined. A dry weight condition index (DWCI) was calculated for each individual and both sexes separately. Further a mean potential increment was calculated for the length classes 21-30, 31-40 and 41-50 mm, provided that a sufficiently large sample size of at least n=20 individuals was given. Since field samples were randomly taken throughout the season and density differed significantly between month and years, 6 illustrative occasions in different years of the time series were chosen, in which density differed greatly within a short time span. These occasions were found, both in recent data and dry weight data from two previously published studies. The data from 2006 and 2007 were collected by Hufnagl *et al.* (2010), and the data from 2010 were obtained from Sharawy *et al.* (2019). Lastly DWCI and potential increment were compared between different densities in the summer month June-October and the years 2018, 2019, 2020 and 2021.

Approach 3 – Analysis of survey data

In the third approach, the length structure of *C. crangon* in commercial fishery- and demersal young fish survey (DYFS) catches was compared between different stations in the German Bight and the years 1997 to 2016. Especially the share of a large length class (61–70 mm) in relation to the next smaller length class (50–60 mm) was analyzed as a potential proxy for growth. Assuming that reduced growth would result in proportionally fewer animals reaching the next larger length class, the absence of large animals relative to many smaller animals should have served as an indicator of poor growth. Subsequently the share of the 61-70 mm length class relative to the share of 51-60 mm length class was compared at different densities. Therefore, survey and fisheries data from the months August to October and years 1997 to 2016. The comparison on the relative abundance of the two length classes was based on DYFS data. Individuals in the total catch were assigned to 10 mm length classes, after their share in the total catch, was calculated based on relative weights of the respective length classes within a subsample. With the aid of a length-weight relationship, the total quantity in kilograms could be calculated into numbers of pieces per length class. The numbers of animals within the different length classes were then normalized to a haul duration of 15 minutes. To check to what extent the survey data represent the overall density in the field, the density calculations (CPUE Survey) were compared to mean landings per unit effort (LPUE) of the commercial fleet as a mean for September and October and for the same regions (ICES squares). Therefore, the percentage share of the two size categories of HCN 1 and HCN 2 out of the landing declaration data was calculated as a mean of both months (September and October) for every year. The catch numbers from the DYFS were converted into catch weight of the commercial fraction (> 6.8 mm CW) per hour.

Size selection of individuals

In the first two approaches, focus was primarily on animals of 20 to 50 mm length since potential growth limitation would only be of interest for individuals that would not be retained by the common fishing gear. In the third approach larger individuals were used in the calculations, since due to the mesh size used in the survey / fishery, only animals larger than 50 mm have a sufficiently high probability of being retained in the meshes of the nets used. If one were to calculate with a smaller length class (e.g., 30- 40 mm) from survey or fishing data, one would run the risk of underestimating the relative quantities in the catch due to higher escape rates.

3. Results

Density

Densities at the sampling site in Büsum ranged between 0.2 (May 2007) to 345.2 individuals per m² (July 2020). In the more distant years of the time series (2006-2010), relatively low densities were observed, with average densities of 1.4-, 0.9-, and 3.3 individuals per square meter for the years 2006, 2007 and 2010 respectively. In the two most recent years of the time series, the highest average densities of 110.9- and 163.6 individuals per m² were found for 2019 and 2020. In all years, density varied greatly between the individual samplings at the sampling site in Büsum, with smaller fluctuations in density being observed in years like 2007 and 2018 and large variation in density in years like 2019, in which density varied by a factor of 153 between months.

Highest densities within one year were observed in summer month, peaking mostly in September. After October densities decreased rapidly and remained low until the end of the respective year. In all years density changes could be observed within a very short time at the catch site. The observed field densities are shown in Table 1 (growth tests) and Table 2 (condition analysis). Density in the DYFS survey data varied largely between subareas and years with values ranging from almost zero individuals to up to 7000 individuals caught in 15 minutes of fishing time which would translate into a density of 0 to 1.7 individuals per m². For a comparison of the scientific survey estimates of shrimp density with commercial LPUE data the mean landings per unit effort (LPUE) of the commercial fleet was calculated for the same ICES-squares as sampled by the DYFS, in each year as a mean of September and October.

Approach 1 - Growth experiments

In total, 2575 first moults were observed, during 24 growth experiments between 2018 and 2021. It was found that the first increment, after remaining comparatively stable over a short period of time, began to decrease if animals had been in the laboratory for longer. The period with constant increments decreased with increasing size of the shrimp (Fig. 4-1). In order to assess the field condition of the animals, a threshold of 4 days for individuals between 21- and 30-mm length (vertical line in Fig. 4-4 A), and 10 days for larger individuals was selected (vertical line in Fig. 4-4 B & C), during which the individuals should have moulted for the first time. Animals that hadn´t moulted prior to this threshold were excluded from this part of the analysis due to possible laboratory effects. 1133 moults occurred in a timespan between catch and this length dependent threshold. The increments, measured immediately after these moults are henceforth referred to as “early increments”. Mean early increments, varied significantly with size of the individuals (Ancova, $p<0.005$), ranging between 0.3 ± 1.3 mm (mean ± SD) in March

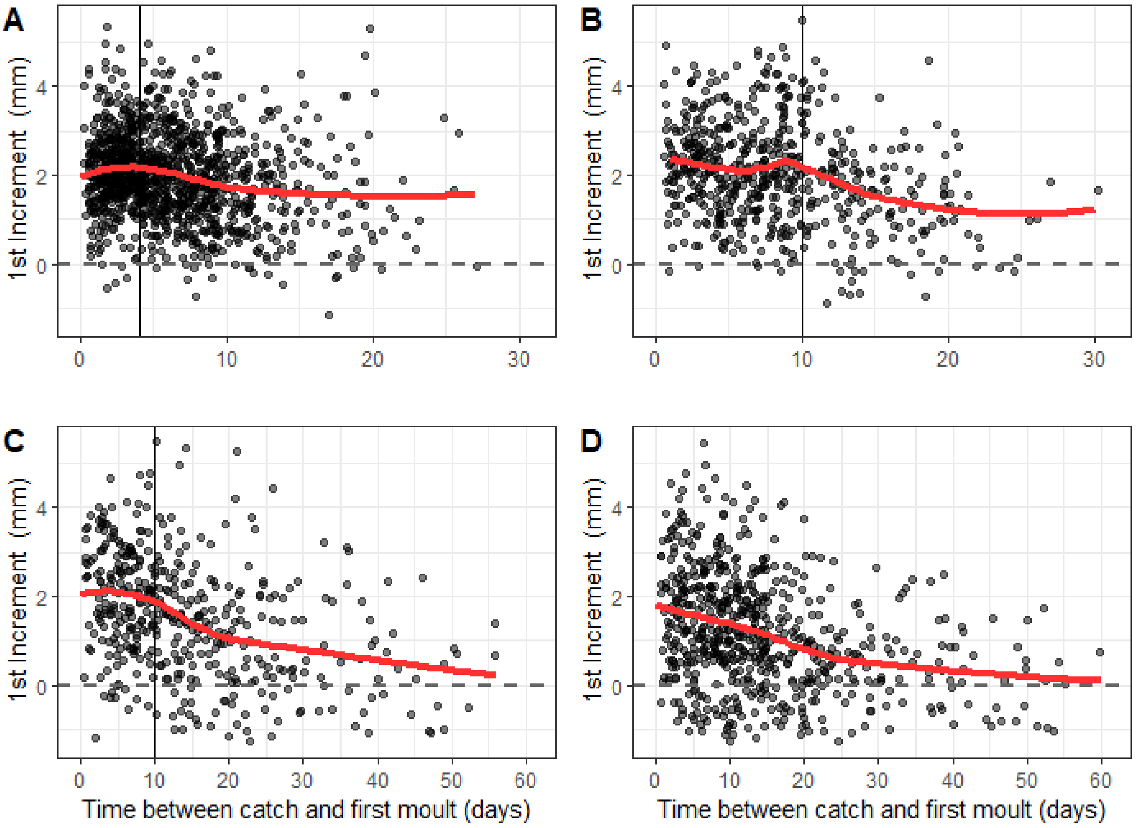

Figure 4--1*: First increment over days in the laboratory. Length classes:* ***A*** *= 21-30 mm,* ***B*** *= 31-40 mm,* ***C*** *= 41-50 mm,* ***D*** *= 51-70 mm*

2019 (length class 41-50 mm) and 3.5 ± 0.8 mm in July 2018 (length class 31-40 mm). In general, smaller average early increments could be observed in individuals sampled in winter.

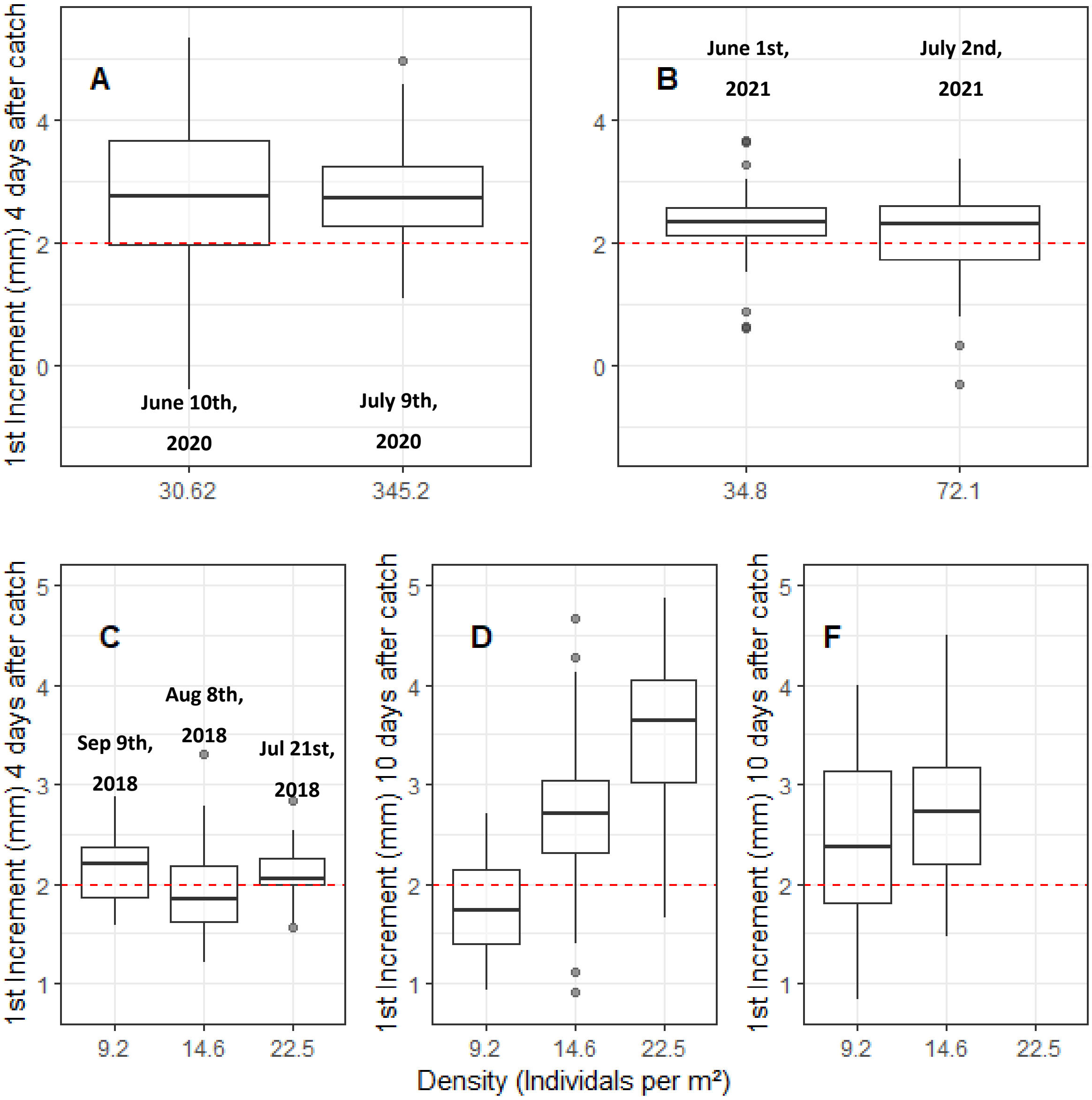

Figure 4-2: *First increment of individuals that moulted shortly after catch, at occasions where density differed by a factor of 11 and 2 in a time span of around 30 days.* ***A****= shows samples of summer 2020 and* ***B****= of summer 2021 both of individuals with length class 21-30 mm. In Plot C, D and F early increments of individuals caught in summer 2018 are shown. In a timespan between July 21st and September 9th (50 days) early increments of length class 21-40- (****C****), 31-40- (****D****) and 41-50 mm (****F****) were compared at three samplings that differed in density by a factor of 2.4.*

Three occasions were identified in which density differed by at least a factor of 2 in a timeframe that did not exceed 50 days. No significant decrease in early increments with increasing density was observed for any of the three selected periods (Fig 4-2.). The two samples from June and July 2020 were about 30 days apart but differed in density by a factor of 11. Nevertheless, the animals of 21-30 mm length showed no significant differences in early increment (Fig. 4-2 A, T-test, p=0.83). Likewise in June and July 2021, where density differed by a factor of 2 early increments of individuals between 21-30 mm turned out to be equal in both samplings (Fig. 4-2 B, T-test, p= 0.48).

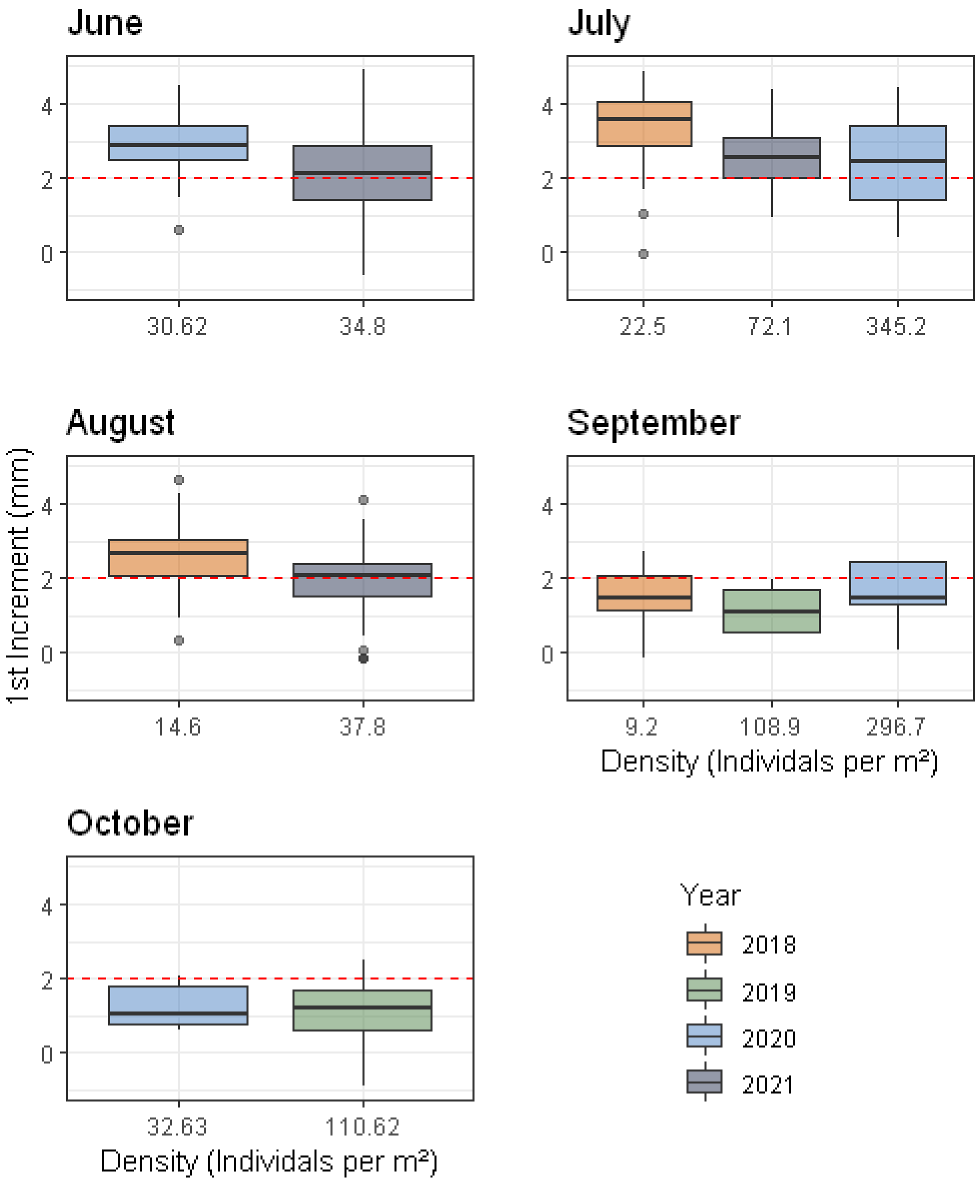

Figure 4-3: *Early increment of individuals between 31-40 mm length during summer month of the years 2018-2021 and at different densities.*

When comparing different length classes in a set of samplings in 2018, that were 50 days apart, it was shown that while the length classes 21-30 and 41-50 showed no significant differences in early increment at the different densities (Fig. 4-2 C – Anova, p=0.632; Fig. 2 E - T-test, p= 0.428), in length class 31-40 mm early increment was significantly larger at higher densities (Fig. 4-2 D - Anova, p<0.001). In addition to comparing the early increment for density changes within a short period of time, it was also compared between different densities of the same month but different years. Fig. 3 shows the early increments individuals between 31-40 mm during the summer months of the years 2018-2021 at different observed densities. While density seems to have a negative effect on early increment in June, July and August, the best average early increments of 1.8 mm in September, were observed in the sample with the highest density (296.7 individuals per m² in 2020). In general, the effect of the sampling year seemed to be greater than that of the density. None of the differences between densities in summer-month was significant (Ancova = 0.672). The mean early increments with the corresponding densities, the trial temperature and the respective catch date are shown in table 4-1.

Comparison of first and second increment in laboratory trials

When comparing the early first increments with the corresponding second increments of the same individuals, it was found that only few individuals showed a second increment that was equal to the first. While in some trials, the majority of the observed animals showed poorer early increments compared to the corresponding second increment (February 2019 at 0.5 Individuals per m²), in other experiments the majority of the animals showed a larger first increment compared to the second increment (e.g., October 2019 at 110 Individuals per m² Inc. 1 = 1.7 ± 0.5 mm, Inc. 2 = 1.0 ± 0.8 mm, Table 1).

No trend towards higher shares of individuals that improved their first increment with increasing density could be found, even if only summer samplings of one respective year were compared (Table. 1). Figure 4-4 A shows the three samples taken in a time frame of 50 days in summer 2018 that differed in density by a factor of 2.4. While at a density of 14.6 individuals per m² (Fig. 4-4, A2) and 22.5 individuals per m² (Fig. 4-4, A3), individuals showed a comparable growth performance in both moults, illustrated by the diagonal dashed red line, the animals at the lowest density of 9.2 individuals per m² (Fig. 4-4, A1) showed a comparably worse second increment compared to the early increments.

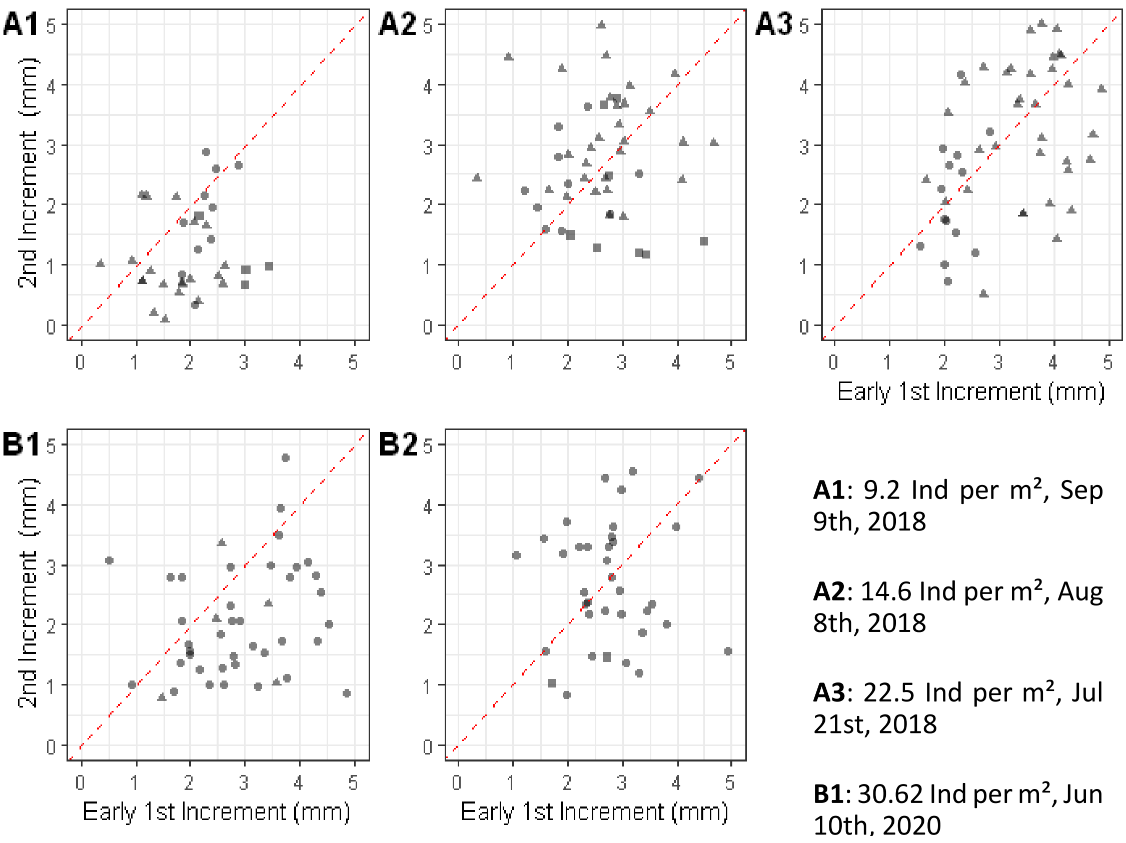

***Figure 4-4**: Shown are first and second increment of the same individuals. Circles = Indidviduals of 21-30 mm length, triangles= Individuals of 31-40 mm length and squares = individuals of 41-50 mm length. The red dashed line indicates a similar growth performance in the first and second moult. A includes three occasions in summer 2018 where density differed by a factor of 2.4 with **A1** = the lowest density to **A3** = highest density. B includes 2 samplings from Summer 2020 that differed by a factor 11.3 in 30 days **B1**= lower and **B2** = higher density.*

Large increments after both the first and second moult were primarily shown by larger animals (triangles and squares Fig. 4-3, A1-3). In the two samplings from 2020, which differed in density by a factor of 11, the animals at the lower density showed better early increments compared to the second increments. At the higher density, the animals with a length of 21-30 mm (points in Fig. 4-4, B2) showed an early increment with comparatively low variability, while the second increment varied significantly more. The two trials from 2021, selected when considering only the early increments, were not included since too few second increments could be observed.

Mortality

Mortality in the experimental trails ranged between 2.5 % in length class 31-40 mm in July 2021 and 68.8 % in September 2018 and length class 41-50 mm. Larger individuals (length class 41-50mm) tended to show with an average of 46.9 %, higher mortality rates than smaller ones (19.5 % and 18.8 % for length class 21-30 and 31-40 mm respectively). When comparing the average mortality of summer (17°C) and winter sampling (11°C) both showed similar average mortalities with around 20 %. Strong differences in mortality could be observed between the different month of one year and between years. 2019 showed with 37% the highest average mortality, while the other three years ranged between 21.5- and 27%. In none of the 4 sampled years increasing mortality-rates could be observed with increasing density when only summer samplings with a temperature of 17 °C were compared. On the contrary, in both 2018 and 2020, the highest mortalities were observed at the lowest density. When comparing mortality at trials that greatly differed in density but were taken in a short period of time no trend towards higher mortalities at higher densities could be observed. While in June and July 2021 (35- and 72.1 individuals per m²) mortality was 31.3 % in both trials, in samplings from 2018 and 2020, mortality differed only marginally between sampling (12.2 % at 30.62 Ind. per m² in June 2020 compared to 13.4% at 354.2 Ind. per m² in July 2020, Tab. 1), or was higher in the samplings with lower densities (46.2% at 9.2 Ind. per m² in September 2018, 13% at 14.6 Ind. Per m² in August 2018 and 18.8 % at 22.5 Ind. per m² in July 2018, length class 21-30 mm, Tab. 1) .

Tab 4-1: *Mortality, first increment (mm), second increment (mm) and sample size (N) for three different length classes (left = 21-30 mm, mid= 31-40 mm and right 41-50 mm)*

Density	Date	°C	N	Mortality after 30 days	Inc 1 in mm 0-4 days after moult (Mean± SD)	Inc 2 in mm (Mean ± SD)	N	Mortality after 30 days	Inc 1 in mm 0-10 days after moult (Mean± SD)	Inc 2 in mm (Mean ± SD)	N	Mortality after 30 days	Inc 1 in mm 0-10 days after moult (Mean± SD)	Inc 2 in mm (Mean ± SD)
0.5	Feb. 2019	12	46	10.9	1.6 ± 0.6	1.7 ± 1.4	17	11.8	1.5 ± 0.5	1.2 ± 1.4	-	-	-	-
3.46	Apr. 2020	11	25	42.4	2.9 ± 1.1	-	39	-	2.0 ± 1.5	-1.4 ± 1.0	17	58.8	-	-
9.2	Sep. 2018	17	26	46.2	2.2 ± 0.4	1.6 ± 1.0	82	46.2	1.7 ± 0.6	0.7 ± 0.8	16	68.8	2.5 ± 0.9	-
9.5	Mar. 2021	11	10	20	1.5 ± 0.5	1.7 ± 1.0	-	23.2	-	-	-	-	-	-
10.4	Nov. 2020	11	41	7.3	2.1 ± 0.2	-	38	23.7	1.4 ± 0.9	-	-	-	-	--
14.6	Aug. 2018	17	23	13	2.0 ± 0.6	2.5 ± 1.0	46	15.2	2.7 ± 0.9	3.1 ± 1.0	21	33.3	2.8 ± 0.7	2.5 ± 1.6
17.6	Oct. 2021	17	-	-	-	-	-	-	-	-	-	50	2.3 ± 1.0	0.4 ± 1.9
22.21	Nov. 2018	12	18	33.3	1.4 ± 1.1	1.5 ± 1.7	26	30.8	1.5 ± 0.4	1.0 ± 0.8	-	-	-	-
22.5	Jul. 2018	17	48	18.8	2.1 ± 0.3	2.1 ± 1.2	47	14.9	3.5 ± 0.8	3.3 ± 1.1	-	-	-	-
29.2	May. 2019	17	85	3.5	2.7 ± 0.6	1.8 ± 1.3	-	-	-	-	-	-	-	-
30.05	Apr. 2019	10	61	3.3	2.2 ± 0.6	1.6 ± 0.9	73	11	2.1 ± 1.3	1.9 ± 1.9	38	28.9	1.4 ± 1.7	-
30.62	Jun. 2020	17	90	12.2	2.8 ± 1.1	1.9 ± 1.2	-	12	2.7 ± 1.2	-	-	-	1.7 ± 1.1	-
32.63	Oct. 2020	17	66	34.8	1.7 ± 0.6	1.1 ± 1.0	12	25	1.2 ± 0.5	-	21	-	1.2 ± 0.7	-
34.8	Jun. 2021	17	45	11.1	2.5 ± 0.8	-	104	21.2	2.3 ± 1.1	-	42	23.8	2.1 ± 1.1	-
35	Jun. 2019	17	90	3.7	2.0 ± 0.6	1.7 ± 1.1	-	-	-	-	10	90	-	-
37.8	Aug. 2021	17	67	6.8	1.8 ± 0.5	1.1 ± 0.9	90	10	2.1 ± 0.8	1.0 ± 1.2	31	-	2.4 ± 0.9	-
38.98	Mar. 2019	10	-	-	-	-	-	-	-	-	-	100	-	-
70.6	Aug. 2020	17	-	-	-	-	-	-	-	-	45	8.9	3.1 ± 1.5	-
72.1	Jul. 2021	17	76	2.6	2.4 ± 0.8	2.1 ± 1.1	80	2.5	2.9 ± 0.9	3.0 ± 1.3	-	-	-	-
108.9	Sep. 2019	17	84	56	1.5 ± 0.6	1.6 ± 1.1	-	-	-	-	-	-	-	
110.6	Oct. 2019	17	75	32	1.7 ± 0.5	1.0 ± 0.8	17	23.5	1.4 ± 0.7	-	-	-	-	-
216.6	Jun. 2020	17	77	14.3	2.2 ± 0.9	2.3 ± 1.3	-	-	-	-	-	-	-	-
296.7	Sep. 2020	17	72	23.6	1.8 ± 0.6	1.9 ± 0.8	-	-	-	-	27	7.4	2.2 ± 0.8	-
354.2	Jul. 2020	17	97	13.4	2.8 ± 0.9	2.5 ± 1.2	-	-	-	-	12	-	-	-

Approach 2 – Analysis of dry weight at length

Dry weight condition index (DWCI) in the time series ranged between 0.90 ± 0.2 in length class 31-40 mm (March 2007 at a density of 0.37 N/m²) and 1.47 ± 0.1 in length class 41-50 mm (July 2010 at a density of 9.4). Neither the comparison of DWCI at different densities withing a short period of time (Fig. 4-5 A-F), nor the comparison of different densities in one respective summer month (all years grouped, App. 3), revealed a trend towards lower DWCI values at higher densities (App. 3-4). While animals in the periods of April 2006 and September 2019 showed the lowest conditions at the highest observed density (Fig. 4-5, A & E), in the periods of July and June 2020 (Fig 4-5. C & F) significantly better DWCI could be observed at the highest density. No trend towards poorer DWCI at higher densities was observed in the periods from June 2007 (Fig. 4-5 B) and July 2010 (Fig. 4-5 D). In general, better DWCI were observed in the summer months of June-October. In particular, the early part of the year, such as February 2006 and March 2007, was characterized by very low average DWCI. In comparison, the best average DWCI were observed in 2010. All DWCI for the different length classes and densities can be found in the Appendix (Table 2).

When comparing potential increment at different densities, no recognizable trends towards better increments at lower densities were found, when only samplings of one respective year were compared. In general, the larger animals seemed to show better increments at a given condition (Table 2). While mean potential increments of 1 to 2.5 mm were calculated for the smallest length class, the increments of the next higher length class ranged between 1- and 3 mm. increments of between 2 and 8 mm were calculated for the length class 41-50 mm. However, the strong differences even within a length class could not be explained by the differences in density. Analogous to the results of the DWCI, both better and worse potential increments were observed with increasing density. The analogies to DWCI notwithstanding, were samples (e.g., length class 21-30 mm, density 1.70 in November 2010) in which the potential increment was again high in relation to a poor mean DWCI.

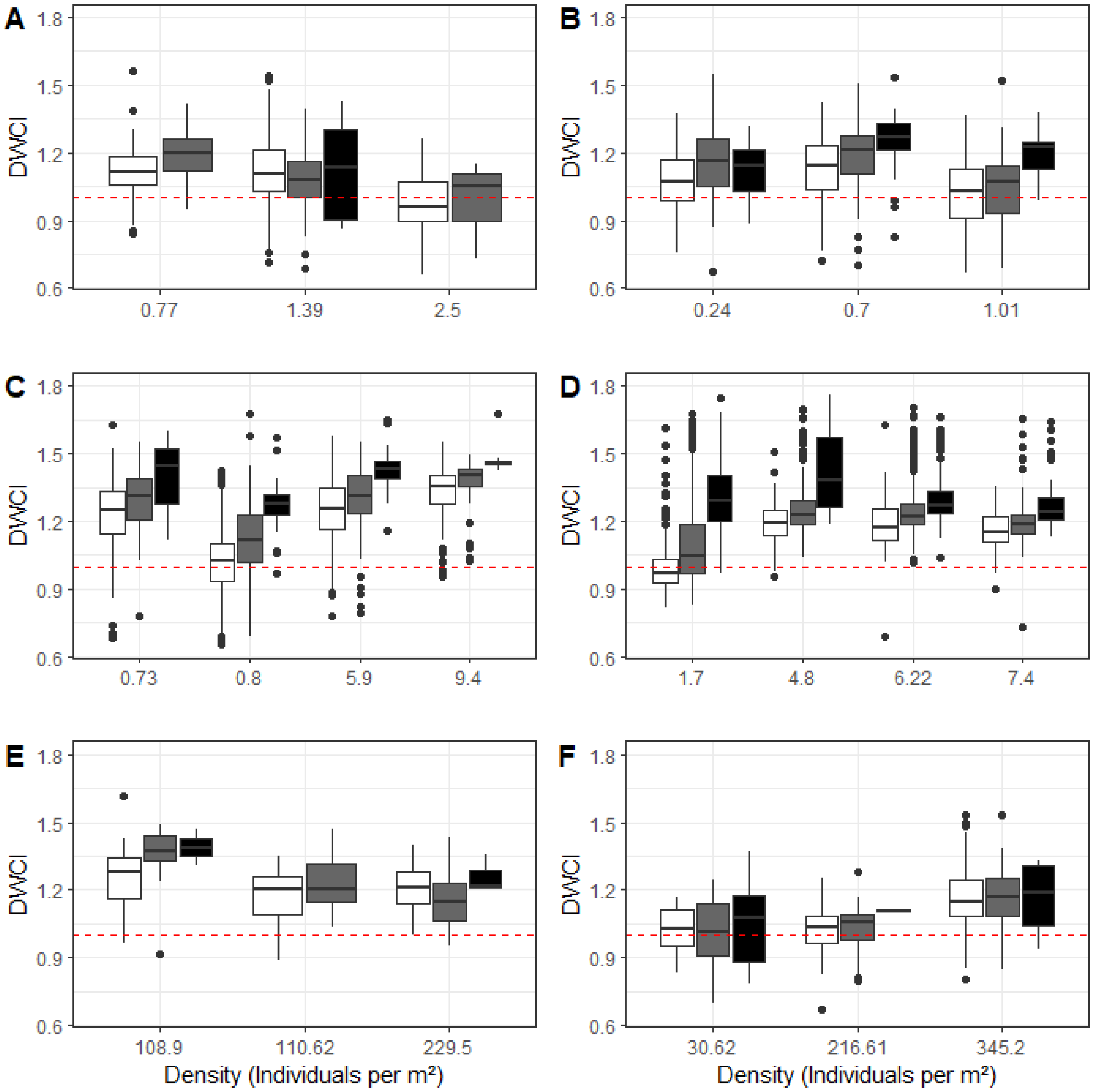

Figure 4-5: *Shown is the comparison of DWCI on six occasions were density differed within a small scale of time. A= Samplings that were performed in April 2006, B= Samplings performed in June 2007, C= Samplings that were performed in July 2010, D= Samplings that were performed between end of October and Start of November 2010, E= Samplings that were performed between September and early October 2019, F= Samplings that were performed between June and early July 2020.*

Approach 3 – Size composition analysis from survey data

When comparing the share of a large length class (60–70 mm) in relation to that of the next smaller length class (50–60 mm) in different subareas of the German EEZ between 1997 and 2016, no clear pattern towards fewer individuals with increasing density could be found (Fig 4-6 A). Although density differed by a factor of 3 between years, higher densities do not seem to have any effect on the size structure of the shrimps. Figure 4-6 A shows the ratio as a percentage of the abundance of the 60–70 m length class relative to the abundance of the 50–60 mm length class for all sub-areas and years plotted over the mean shrimp density (N /15 min) while in Fig. 6B subareas are combined into a single year value. In both cases the proportion of 60-70 mm large animals in relation to the next smaller length class varied between 25 and 60 % without a decreasing trend although densities differed by a factor of 3 between years (Fig. 4-6).

The comparison of density data from the DYFS, to patterns that emerge from the activity of the whole shrimp fleet revealed a linear relationship between CPUEs from the survey to LPUEs from commercial fishery date at the same time and in the same region. Figure 6C shows the commercial LPUE values plotted against the corresponding catch weight of the commercial-sized shrimp from the DYFS data of the same area and year. While there is a general positive correlation the data show a wide scatter and also some systematic bias. In certain stations and years (e.g. 2005) the survey tends to underestimate the commercial LPUE, probably due to an offset between the survey and the commercial fishing positions. Further dose regression line between LPUEs and CPUEs not intercept at the origin, which implies, that the commercial landings overestimate the true density in years with low shrimp biomass. If the data are averaged for the whole year the scatter is much reduced, but the pattern is similar with a positive intercept (Figure 4-6, D).

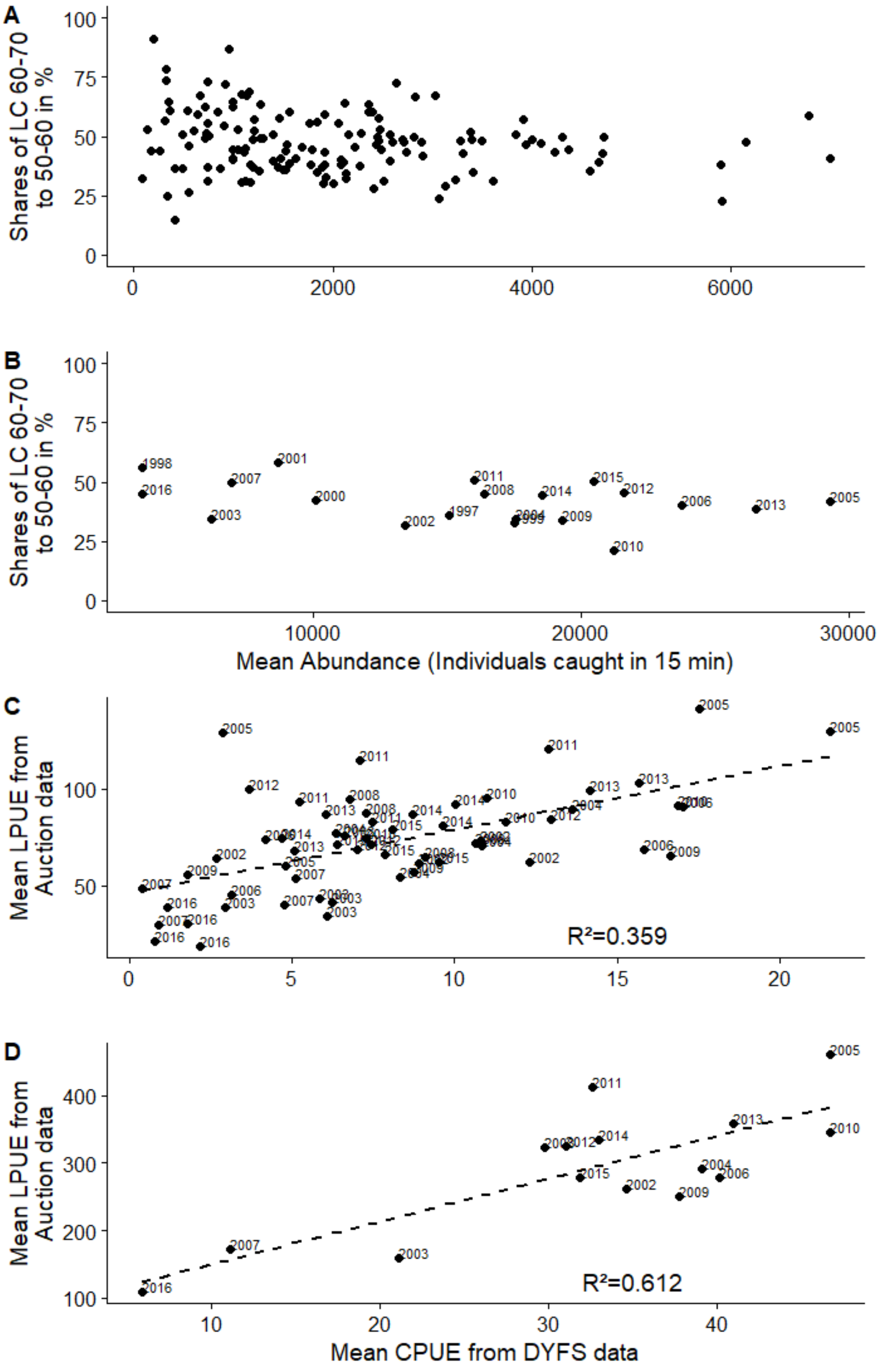

***Figure 4-6**: Shown is an analysis from the 2018 ICES WGCRAN report, investigating the potential growth limitations by density (A & B), and the comparison of commercial fishery data to survey data of the DYFS (C & D) in the years 1997 to 2016 in the German EEZ*

4. Discussion

Seasonal differences in density on a small scale

Density, on the sampling site in Büsum varied largely between different months of one year. These differences, occurring within a short period of time and in such a locally restricted dataset, should primarily be determined by drift and migratory behavior as well as the overall recruitment level (Temming & Damm, 2002; Hufnagl *et al.*, 2014). In all years, differences in density were observed between the winter months, early summer, and the summer months. While few animals were found on the tidal flats in winter, their density increased massively over the course of the summer, only to reach a lower level again in the following autumn. These observations are in line with existing knowledge on the annual migratory behavior of the species, which can presumably be cited as the cause for both the local size composition of the catch, as well as the absolute number of animals caught (Boddeke, 1976; Penning *et al.*, 2021). High densities in late spring and summer are driven by two factors. On the one hand, the annual migration of adults between deep, warmer habitats in winter and shallow, food-rich mud flats in summer leads to adults appearing in shallower water by the onset of spring (Boddeke, 1976). On the other hand, newly arriving recruits by the end of May/ early June lead to a doubling or multiplication of the densities (Kuipers & Dapper, 1984; Cattrijsse *et al.*, 1997; Temming & Damm, 2002). As summer progresses, new recruits continue to arrive from spring- and summer egg productions and the earlier arrivals grow larger while moving gradually to deeper waters. Later between October and November, a strong decline in density is apparent in almost all years of the time series. Mature shrimp begin to seek deeper-, often warmer water layers and start to leave the coastal areas (Hartsuyker, 1966). Depending on the temperature development in the years, this event may occur earlier or later, which could explain shifting density peaks between the years. Few individuals remain on the mudflats until winter resulting in the lowest densities observed in the following spring. In addition to the water temperature and the life stage of a shrimp, the respective food situation in the habitat can also trigger migration. Unfavorable conditions, such as a deterioration in the food situation will inevitably lead to a reduction in local density, as the animals start to migrate towards other habitats.

Density values reported for *C. crangon* vary greatly and depend strongly on the respective habitat investigated. Published figures vary from 0-5 individuals per square meter in adult habitats to up to 100 (N/m²) on nursery grounds (Henderson *et al.*, 2006; Campos *et al.*, 2009; Penning *et al.*, 2021). In contrast the observed densities of more than 200 Individuals per m² at the sampling site in Büsum clearly exceeded these values several times between 2019 and 2020. One reason for this could be the unique location of the sampling site. The proximity to the fairy way of the Büsum harbour connects deep water with shallow water on the tidal flat and thus reflects an area that is both suitable for juveniles and adult shrimp. The funnel-like inlet of the harbour entrance aggregates the animals at low tide within a radius of about 2x2 km to a comparatively small body of water. Since due to reachability of the sampling site, all the samples were taken at low tide and therefore at a timeframe of greatest aggregation. Nevertheless, even assuming a more homogeneous distribution of individuals at high tide, the observed densities would probably have been high compared to literature values.

Inter-annual differences in density

Density, both on the sampling site in Büsum and the DYFS and commercial fishery data also varied greatly between the different years. General abundance of common brown shrimp within a year is likely to be driven by the strength of incoming recruitment waves, which in turn is determined by external factors, influencing mortality in all live stages of the shrimp. In addition to the size of the adult population, which ultimately determines the number of eggs in a season, factors such as food availability, predator presence, diseases and fishing pressure can influence absolute number of animals within the population. While formerly the population size was strongly influenced by the presence of the two main predators cod and whiting, nowadays mainly the fishery is believed to be the main driver of mortality in the common brown shrimp stock and hence the population size (Hufnagl & Temming, 2015; Tulp *et al.*, 2016). A recent study found a correlation between the winter fishery for adult, presumably egg-bearing, shrimp off the coast of the Netherlands and shrimp densities off the north-Frisian coast the following summer, indicating local recruitment overfishing (Respondek *et al.* 2022). However, an overall reduction in fishing effort in certain regions of the North Sea, due to catch restrictions imposed by the processing industry during the corona pandemic did not lead to corresponding increase in the shrimp stocks in these regions (ICES, 2022). Together with extremely high population densities in 2018 and 2019, this leads to the conclusion that some of the main factors determining population size are rather bottom up driven, operating via larval survival and/or optimal growth conditions.

Growth at different densities

Besides moulting interval, moult increment is one of two factors determining growth rate in *C. crangon*. Hence its analysis should have given insight on at least one part the growth potential of an individual. The analysis of increments of early post-capture moults was based on the assumption that an early increment should reflect the prevailing food situation in the field. This assumption supports a method frequently used in crustaceans to calculate in situ growth increments. In the instantaneous growth rate (IGR) method, as the name implies, increments of moults, observed immediately after capture are averaged to calculate in situ growth increments in different length classes of one respective species (Quetin, L. B., Ross, R. M., & Clarke, 1994). Therefore, only increments that could be measured up to a certain point after capture were analyzed. Growth performance in *C. crangon* and hence growth increment is known to vary significantly with factors such as, length, sex, temperature, food availability and quality and seasonal origin (Hufnagl & Temming, 2011a). The influence of the individual factors on growth was analyzed in a meta-analysis of data by various authors summarized in 2011 by Hufnagl & Temming. All factors but seasonal origin can be excluded as potentially masking, density effects, since sex ratio was excluded statistically and trial temperature, size and food availability were set equal in laboratory trials. Based on the observations of Hufnagl and Temming (2011a), in which winter egg recruits were hypothesized to be primarily responsible for good growth rates, it can be assumed that the cohort effect could serve as an explanation for observed differences. While this seems reasonable when comparing a length class over a longer period of time, selecting samplings within a month or a short period of time prevented potential mixing of different cohorts.

After analysis of the first increments, its improvement should have served as a measure for potential food limitation due to high density. Temporary food shortages, even in a generalist feeding type species like *C. crangon*, and in a highly productive ecosystem such as the southern North Sea can indeed occur. According to Hufnagl *et al.* (2010), periods of starvation are found primarily in winter, although, there are indications that a certain percentage show signs of starvation even in periods when most animals are in good nutritional condition (Perger & Temming, 2012). Based on the assumption that the early increment reflects the field situation, while the second increment, after days of ad libitum feeding, represents growth under "good feeding conditions", both increments were compared. Here, especially for situations in which a large proportion of the observed animals showed a strongly improved growth performance in the second increment were of interest, as this could indicate a poor nutritional condition under previous field conditions. The comparison of first and second increment showed, that in few cases ad libitum feeding can improve the growth potential of the animals. However, in the majority of the samplings, the variability in both the early and the second increment did not allow for a clearly discernible improvement in growth potential. The fact that in almost all samplings, a higher variability in second increment could be observed compared to early increments, showed that the time between first and second moult in captivity can have different effects on the individuals. While some individuals obviously benefit from being in captivity, others seem to grow rather worse. It cannot be excluded that stress induced by capture, husbandry and daily monitoring could have negatively affected the animals (Lagadére, 1982; Stoner, 2012; Delbare *et al.*, 2015). It should be noted that only rarely was a situation found in which almost all animals grew poorly in the first increment and better in the second increment, which suggests that at no time in summer was there a severe food limitation in the field.

In addition to moult increment, another measure for growth which is advantageously not influenced by the husbandry in the laboratory, was the average dry weight at a given length. Dry weight at length in *C. crangon* is subject to constant change, since the build-up of dry matter and the regular translation of it into growth is part of the regular moulting cycle (Hartnoll, 2001; Perger & Temming, 2012). At the same time, however, it is known that food limitation, which can be a result of intraspecific concurrence, also affects dry weight condition. In the case of starvation, the animals lose dry mass at constant length and their DWCI decreases (Hufnagl *et al.*, 2010b). For this reason, and under the assumption that high densities lead to food limitation, occasions in which low DWCI were observed at high densities were of special interest. Neither in the comparison of DWCI at different densities, nor when comparing potential increments, a trend towards decreased condition / growth was observed with increasing density. On the contrary, the often-best DWCIs and highest potential increments could be observed at the highest densities. This is consistent with results from Hufnagl *et al.* (2010) in which they found a positive correlation between density and DWCI. Since higher densities were primarily observed in summer, when generally a higher presence of potential prey for *C. crangon* can be found, higher condition values at higher densities might mainly be an artefact of seasonality. To extrapolate the observed growth differences of animals from

Büsum to other habitats, such as deep spawning grounds off the East Frisian Islands, risks neglecting habitat differences in density and food availability.

The final, less regionally applicable proxy for common brown shrimp growth was thus a comparison of the relative proportion of two successive length classes in survey data at different densities. Although density differed by a factor of three between years, the share of the large size class (60-70 mm) relative to the smaller size class (50-60mm) was highly variable but without a clear relationship to density. The comparison of survey and fishery estimates revealed a close correlation with a slight overestimation in population size, especially in poor years when only commercial fishery data was used. However, since the fishing operations involve the whole area of the southern North Sea and a much larger sampling volume, these should give the most representative picture of population density. Analogies can be found between the DWCI and the length structure in the survey data. If one selects the years 2007 and 2010 as examples, it is noticeable that in terms of density at the sampling site in Büsum in 2007, for example, densities were similarly low as in the survey data. At the same time, the animals in 2007 showed a relatively low DWCI and low potential increments, regardless of the month in which they were sampled. This perceived low growth potential is also reflected in the poor 2007 population density (LPUEs). In comparison, both survey/commercial fishing data and the samplings from Büsum in 2010 show higher density values. The growth potential, based on the DWCI calculations, was also significantly higher in 2010, and is thus also reflected in the total population size.

Mortality

In the majority of the growth trials after 30 days, a mortality of 10 to 30 % was observed. This is consistent with the results of other authors where mortality was also in this range and varied from trial to trial (Oh & Hartnoll, 2000; Hufnagl & Temming, 2011a). Lowest mortalities were observed in trials that were run at lower temperatures. Mortality is known to positively correlate with temperature in brown shrimp. One can assume that with low feed intake, a higher temperature, with faster metabolism, leads to faster consumption of energy reserves. The diseases that multiply more strongly at higher temperatures can, in combination, lead to higher mortality at higher temperatures. Although C. crangon can cope with a wide range of temperatures, its thermal preference lays around 16° (Reiser *et al.,* 2014). Higher mortality values coincided with samplings in which on average larger individuals were stocked. This is consistent with findings of Hufnagl & Temming (2011a) were larger individuals showed a lower tolerance to higher temperatures. Despite the differences in mortality between the samplings, no trend towards higher mortalities at higher densities could be observed.

Conclusion

In three different approaches, proxies for the growth of common brown shrimp, at different densities, were investigated. None of the three approaches revealed a trend towards reduced growth capacity at increasing density. This was not the case when growth capacity at different densities was examined within a month and grouped by years, nor when growth capacity at different densities was examined within a short period of time. It appears that good nutritional conditions correlate rather with high densities, than high density worsening food conditions. Migratory behaviour of the shrimp, in situations when food conditions worsen, would inevitably lead to a reduction in local density. Vice versa, would a locally restricted increase in productivity lead to an accumulation of shrimp from surrounding areas and to an increase in density. If this idea is applied to a larger scale, and to the population as a whole, it would mean that a productive year would allow many recruits of the common brown shrimp to survive, and thus also high densities. This idea is supported by the fact that all life stages of this very short-lived animal are dependent on primary production (Kuipers & Dapper, 1984). High primary production leads to lots of plankton and hence optimal survival conditions for larvae and juvenile shrimp. Further would a strong primary production support the development of detritus - food for benthic organisms- and hence the survival probabilities of also older specimen of the common brown shrimp. Hence, high densities should be seen as a positive sign, rather than a potentially growth limiting factor. Local abundance as well as population size could therefore be considered bottom up regulated. Furthermore, it was shown that the natural variations in density, both when using data of only one sampling site and based on commercials or DYFS Survey data, far exceeded the expected increase in density of a management measure such as a mesh size increase from 20 to 26 mm of around 23%. It can therefore be assumed that the growth rates of brown shrimp will not be affected because of a mesh size increase during summer. In winter, when the condition of the animals is generally lower, it cannot be excluded that an increase in density would lead to a further deterioration of the condition. This would also explain why other others observed increased mortality over winter at higher stock sizes

(Henderson *et al.*, 2006). Found for the Bristol Channel, winter could, with a relatively consistent food availability, determine the amount of surviving shrimp also in the southern North Sea. Although this theory could be backed by relatively constant LPUE values of the fleet in the first quarter (ICES 2021) of the last 10 fishing years, this could not be confirmed do date. Apart from this, it is also not advisable to increase the input to counteract the density-dependent effects that occur in winter. The animals are in poor condition at this time anyway and should not be exposed to further stressors.

Future studies should look more closely at the influence of external factors on the natural fluctuation in animal density rather than examining density-dependent effects. The results of previous studies (Hufnagl & Temming, 2011a) and the comparison of individual length classes in successive months in the present study, showed evidence of the existence of a cohort that consistently shows high growth potential throughout the year, while several other cohorts that recruited earlier or later in the year are characterized by poorer growth potential. Suggesting, that the seasonal influence, as well as the seasonal origin of the individuals, have a significantly greater impact on the fitness of the animals than density.

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Manuscript 3: Cohort Effect in common brown shrimp (*Crangon crangon*) – Differences in growth potential between shrimps of different seasons

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Growth, *C. crangon*, cohort, seasonality, winter eggs, summer eggs

Abstract

The common brown shrimp (C. crangon) is one of the most valuable crustacean species in the North Sea, by providing both the basis for a large and valuable fishery, as well as important ecosystem services, by connecting the different trophic levels North Sea food web. Since cohort tracking and age determination in common brown shrimp is close to impossible due to an extended spawning period and the lack of moult enduring hard structures, growth to date remains the only key figure in forecasting stock developments and establishing management measures. Although growth in common brown shrimp has been subject of investigation of many studies to date, variability in figures between the studies remained high and largely unexplained. A factor potentially influencing growth, first introduced in 2011 is the so-called cohort effect, according to which different cohorts of the common brown shrimp are said to show different growth potentials. Within the present study we investigated the effect of seasonality of same sized individuals on their growth potential. During elaborate growth experiments we found that winter egg cohorts, consistently

1. Introduction

The common brown shrimp (*Crangon crangon*) is considered a key species in the southern North Sea ecosystem. By being both predator and prey for a variety of other species, it links the various trophic levels, and enables a vast energy turnover in one of the most productive ecosystems in the world (Pihl & Rosenberg, 1984b; Pihl, 1985; Jönsson *et al.*, 1993; Van Der Veer *et al.*, 1998). Besides its ecological value, *C. crangon* is also one of the most valuable crustacean species caught for human consumption in the southern North Sea, with a 500 vessel strong fishery yielding a total annual value of around 120 million € (STECF, 2020). Despite its ecological and economical value, the species receives little political attention, and was lacking a management until 2016, when the fishery implemented a self-management during the certification process of the fishery by the marine steward ship council (MSC).

Since a year- round spawning period (Boddeke, 1981; Temming & Damm, 2002; Campos *et al.*, 2009; Hünerlage *et al.*, 2019) and the lack of moult-enduring hard structures, make age determination in *C. crangon* nearly impossible (Hartnoll, 2001), age-based management approaches are not applicable for this species. Hence, many of the measures, implicated in the self-management plan were based on the diagnosis a population specific yield per recruit simulation (Y/R) model (Temming *et al.*, 2017) in which growth rate estimates of Hufnagl and Temming (2011b) were used to simulate the outcome of measures, like a mesh size increase or effort reduction (Günther *et al.*, 2021). Since all subsequent conclusions from the model simulations are highly dependent on the actual growth rates used, understanding the mechanisms influencing growth in *C. crangon* is key for the development of future management measures and a reliable prediction of outcomes.

Growth in the common brown shrimp is dependent on various factors that have been identified over decades of growth research on the species (Hufnagl & Temming, 2011b). Five major factors were summarized in a previous study, which included all findings on growth of *C. crangon* to date (Hufnagl & Temming, 2011a; b). Using ad libitum feeding regimes, four of the five factors, temperature, sex and size were widely studied, and are therefore fairly well understood, the fifth factor has only been mentioned once in literature (Hufnagl & Temming, 2011a), and to date no further studies investigated the influence of the so called “cohort effect”. In 2011 (a) Hufnagl and Temming found that two groups of individuals of the same size grew very differently although they were sampled only few weeks apart. They found that individuals of

20-30 mm length caught in May, showed poor to almost no growth, while individuals of the same size caught in July of the same year, had grown much better, even exceeding all growth observations documented up to this point in time. One possible explanation was, that July individuals were fresh recruits, hatched from winter eggs, while individuals sampled in May were most likely overwintered summer egg recruits, and had experienced long periods of poor food availability (Hufnagl *et al.*, 2010b).

In the present study, a set of growth experiments from the years 2018-2021 as well as dry weight condition data from an incomplete time series between 2006 and 2021 were investigated, to analyze growth performance differences of same sized individuals of *C. crangon* within the season. In 2012 Perger & Temming found a strong relationship between dry weight condition index (DWCI) and the subsequent growth increment, making DWCI a predictor for growth in *C. crangon*. Further they developed a method, to calculate potential growth based on dry weigh at length. These calculation in combination with the actual measured growth rates from laboratory experiments, were assessed and conclusions in the context of the current self-management plan of the brown shrimp fisheries were drawn.

2. Material and methods

Growth experiments

To investigate the influence of seasonal origin on the growth capacity of the common brown shrimp, a series of growth trials was conducted between late 2018 and 2021. For this purpose, animals of four length classes (21-30 mm, 31-40 mm, 41-50 mm, and 51-70 mm) were transferred from a catch site in Büsum to the Institute for Marine Ecosystem and Fishery Sciences in Hamburg and were studied there for a period of approx. 30 days under ad libitum feeding. A pushnet was used to catch the animals at low tide in Büsum and they were then transported to Hamburg in ventilated 30-liter containers. After an acclimatization phase of approx. 24 hours, the animals were separated, measured, and transferred to their individual compartments, which were connected to a recirculation system. The individuals were fed ad libitum with nauplii of *Artemia salina*, pieces of *Clupea harengus* and *Mysidascea spec*. two times a day while feces as well as left-over food was removed from the tanks. From the measured starting length (TL1) and the respective lengths after the first (TL2) and second moult (TL3), the moult increments were calculated. The trial temperature had to be adjusted to be close to the respective field temperatures. A uniform temperature of 17°C was chosen for the summer months (Mid May- October) of all years for the sake of comparability. Winter month (November- April) were conducted using a temperature between 9- and 12°C, so that at no time the experimental temperature deviated more than 6.5°C from the temperature in the field. The light regime was always 12h/12h (day/night). The first and second increments, as well as the moulting interval of the animals were then compared between the different length classes, months and years of the trial period.

Length, dry weight and sex

Length in experimental trials was measured as total length (TL1) from the tip of the uropodes to the tip of the scaphocerite, both at the beginning of a trial and after each moult (TL2, TL3). Measurements were taken manually first. To minimize stress during the measurement process, a lateral picture, taken shortly after each moult, was later used, to measure length more precisely using the Image editing and processing freeware, Image J. Length of individuals caught for dry weight condition analysis was measured as total length to the nearest millimeter from the tip of the uropodes to the tip of the scaphocerite, using graph paper. Dry weight (DW) was determined after 24 h freeze drying (scale: Satorius ± 0.0001g). Sex was determined from the endopodites of the first and second pair of pleopods and by the presence or absence of an appendix masculina using a binocular. To minimize handling stress during the growth trials, sex was not determined prior to the experiment, but after the end of the experiment, on thawed animals.

DWCI and potential Increment

As a second measure of growth, dry weight condition index (DWCI) and potential increment after Perger and Temming (2012) and Sharawy *et al.* (2019) were calculated for field caught individuals grouped by length class and sampling months for the years 2006, 2007, 2010, 2019 and 2020. Data from other authors and previously published studies were included for this purpose. Dry weight condition data from 2006 and 2007 originated from Hufnagl *et al.* (2010), and the data from 2010 was used in a study by Sharawy *et.al* (2019). However, as the catch and processing of the samples did not differ between the studies, the methodology is explained below for all studies and sampling years together. Field caught individuals for dry weight analysis were transferred to Hamburg on crushed ice and frozen immediately after arrival on -

40°C. They were further stored at -40 °C until being further processed. After thawing and blotting the individuals dry on tissue paper, length, sex, and dry weight were determined for each individual. A dry weight condition index (DWCI) was calculated as:

$$DWCI = \frac{DW}{a \times TL^{b}}$$

With DW being individual dry weight and TL being total length, using the sex specific coefficients a= 0.00394/ 0.00199 and b= 2.72/2.92 for males and females respectively (m/f) from Sharawy *et al.* (2019). The coefficients were used to describe a condition immediately after moult. Hence should a DWCI of 1 represent a post-moult condition, so that DWCI values < 1 could be seen as an indicator for food scarcity or starvation. Further a potential increment was calculated after Sharawy *et al.* (2019):

$$INC_{p} = TL \times \left(\frac{DW - EW}{a \times TL^{b}}\right)^{1/b} - TL$$

With DW being individual dry weight, EW being exuvian dry weight and TL being total length. Exuvian dry weight was calculated using an exuvian dry weight length relationship also provided by Sharawy *et al.* (2019):

$$EW = 0.000523 \times \, TL^{2.89}$$

For subsequent calculation of the mean potential increment within a length class from field samples, the mean of all positive potential increments (DWCI >=1) was multiplied by the factor two (Perger & Temming, 2012). Following the assumption, that the field sample will contain all moult stages, from freshly moulted individuals with minimum DWCI (INCp=0) to individuals at the very end of the intermoult period (INCp=true field increment). Hence the mean of all these will only represent the half true field increment. See Perger and Temming (2012) for more details on the field application, which is not the focus of our study here. The growth proxies calculated in this way were then grouped by month within a fishing year.

Mortality

Mortality between the individual runs of the growth experiments was compared by means of a mortality probability according to Kaplan Meyer, after 30 days (the usual duration of the experiment). Only animals that died during the experiment for unknown reasons other than problems with the circulatory system, handling, or jumping out were considered.

3. Results

Growth experiments- Frist increment

First increments of individuals in 10 mm length classes varied significantly between months within one year when only individuals of one respective length class were compared (ANOVA, p<0.001). The largest average increments of 3.8 ± 0.3 mm were shown by animals in the 41-50 mm length class in July 2018 (Fig 5-1 C). While the mean increments of the two smaller length classes ranged from 2-3 mm in the summer months, the increments of the largest observed individuals ranged from -1 to 2 mm. Within each length class, a strong seasonal effect of the size of the first increment could be observed, so that the animals in the smallest length class (21-30 mm) showed their largest growth increments in the months of May and June while in the following months, significantly decreased first increments were observed (ANOVA, p<0.001, Fig. 5-1 A). With increasing animal size, this peak in the first increment shifted further back in the season. Individuals sampled before or after this peak showed significantly worse increments in all three ascending length classes (ANOVA, p<0.001).

Differences in increment could be observed not only between months within a year, but also between years (ANOVA, p<0.001). This was mainly due to the consistently poorer increments in 2019, in which the animals of all length classes performed rather worse than in the other years. The graph also shows that with increasing animal size, the range between minimal increments and maximal increments increases. The largest length class showed an increasing number of animals showing a negative increment after the first moult, i.e. they shrank after the moult. In general, only in late summer (between August and October) consistently positive increments could be observed in the length class 51-70 mm.

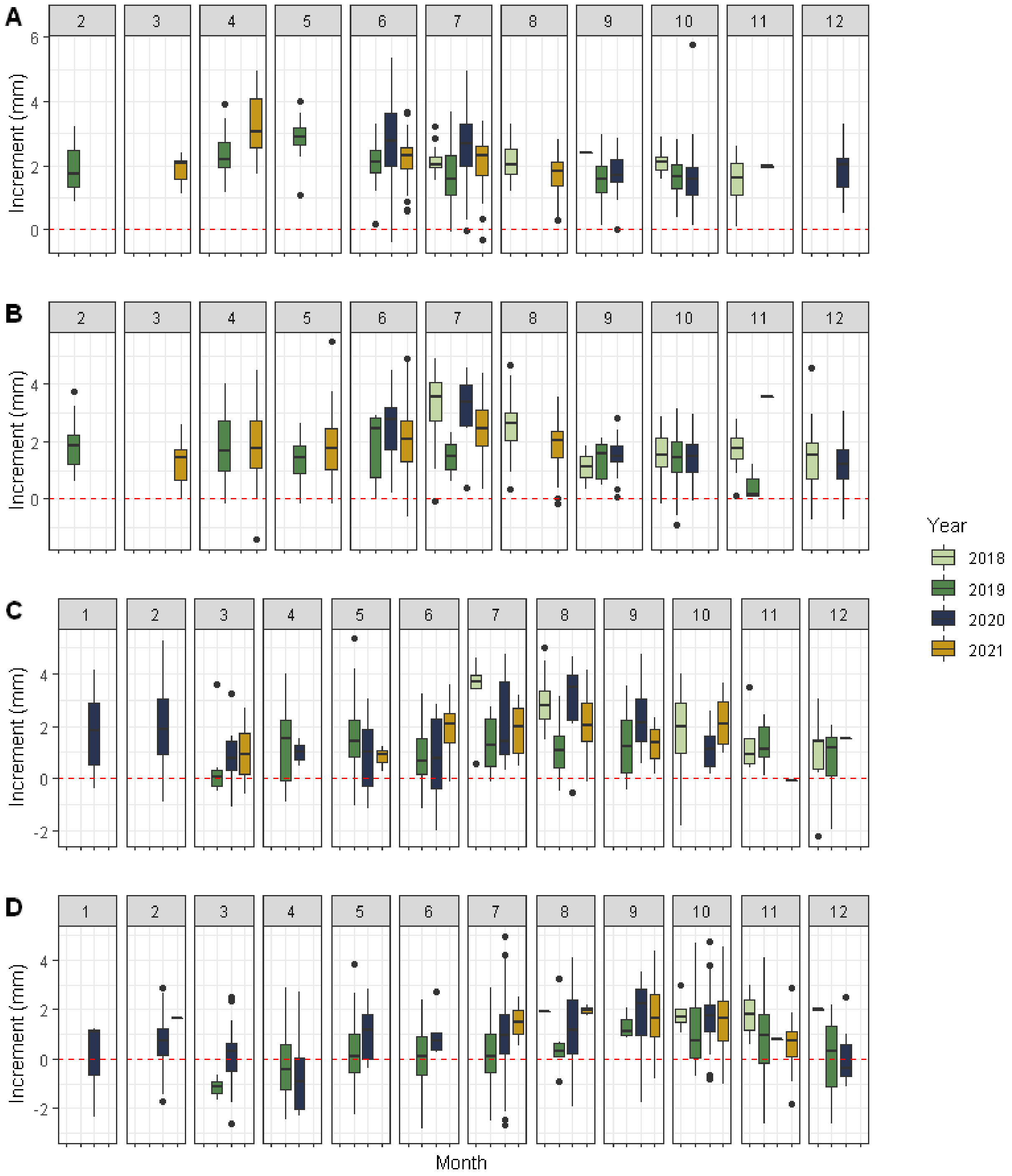

Figure 5-1: First Increment (increment after catch) over month of the sampling years 2018 (light-green), 2019 (green), 2020 (blue) and 2021 (yellow). Shown are 4 different length classes, with A= individuals between 21-30 mm with a selection in increments that were observed latest 6 days after catch, B= all first increments from individuals between 31-40 mm, C= individuals between 41-50 mm and D= individuals between 51-70 mm

Moult Interval

The average timespan between two subsequent moults varied significantly between the different length classes and ranged from 10.5 ± 1.4 days in length class 21-30 mm in July 2021 to 32 ± 4.8 days in length class 51-70 mm in October 2020 (ANOVA, p<0.001).

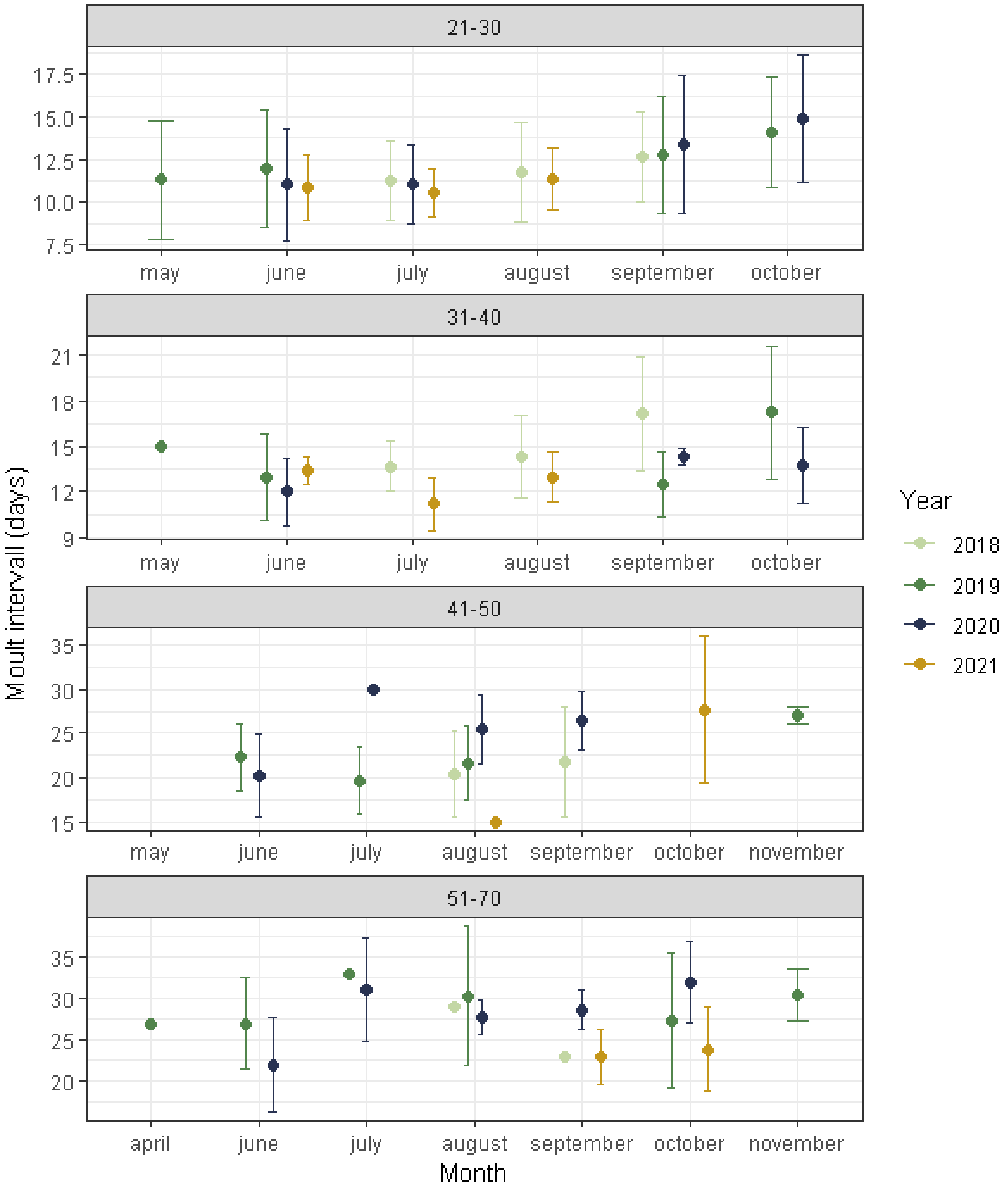

Figure 5-2*: Moult interval at 17 °C of the investigated length classes (21-30 mm, 31-40 mm, 41-50 mm and 51-70 mm), the months May to October and the years 2018 to 2021*

In figure 5-2, only moulting intervals from summer months and the years 2018 to 2021 with a respective trial temperature of 17 °C are shown. Similar to the results for the first increment, the individual length classes seem to show their peak growth performance (shortest intervals) at different times during the summer. Thus, animals of the length class 21-30 mm showed the shortest average moulting intervals in the months of June and July with an average of 10 to 12 days, while just towards the end of the summer, in the months of September and October with around 13 to 15 days, significantly longer moulting intervals were observed (ANOVA, $p<0.001$). With increasing animal size, the performance peak shifts backwards in the season. While the signal is still visible in the length class 31-40 mm with the shortest moult intervals of around 13 days in July to August, it disappears in the next larger length class. Length class 51-70 mm showed with around 25 days the shortest mean moult intervals in September, and thus in the same period as the largest mean growth increments.

Growth rate

The combination of moult increment and moult interval, the growth rate, expressed in mm per day, within the season and grouped over all sampled years (2018-2021) is shown in Fig 5-3, A-D. Ascending by length class (A=21-30 mm and D=51-70 mm), the combination of first increment and the period between two moults once again illustrates the significantly changing growth performance of the individual length class over the course of the season (ANOVA all length classes, $p > 0.001$). Combining all sampled years, individuals between 21-30 mm, grew with mean growth rates of around 0.3 mm best in May. In June and July, growth rates were still high at an average of 0.23 and 0.25 (for June and July respectively). From then on, the growth rates decreased continuously until the end of the season, reaching a minimum of 0.02 mm per day in December (Fig. 5-3 A). Individuals between 31-40 mm lengths (Fig.5-3, B) grew best in July with 0.28 mm per day. While in June and August the animals also showed comparably good growth rates of around 0.2 mm per day, growth in the month before June and after August was poor with 0.1 mm per day or less. In length class 41-50 the growth peak appeared in August with approx. 0.2 mm per day (Fig. 5-3 C). The growth of the largest length class was generally worse and best in September with 0.1 mm per day (Fig. 5-3 D).

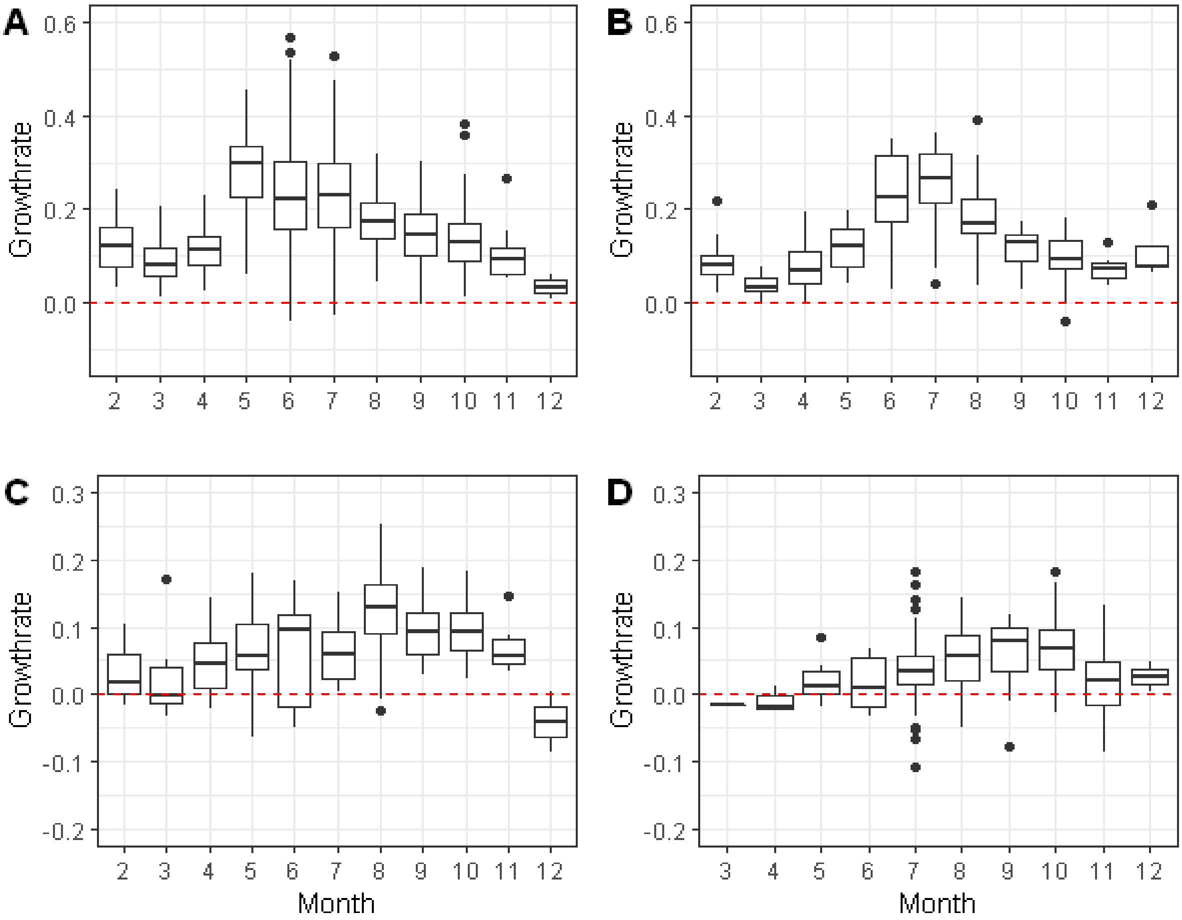

Figure 5-3*: Growth (mm per day) rate with first increment and moult interval after second moult, with A= individuals between 21-30 mm, B=individuals between 31-40 mm, C= individuals between 41-50 mm and D= individuals between 51-70*

Keeping individuals separately over the period of two successive moults allows an observation that to date, has not been documented before. By contrasting the moulting interval and the corresponding increments of single individuals, a completely new way of looking at the growth rate calculation is made possible. In Figure 5-4 it becomes apparent, that although all animals were kept under the same conditions (17°C/ ad libitum feeding regime), they differ strongly in their growth potential even within one experimental run. Well growing animals are characterized by a short moult interval and large growth increment, this combination results in a high growth rate. Poorly growing individuals, on the other hand, show small growth increments with a long moulting interval. However, the average growing individual did not show the combination of long moulting interval and high growth increment at any time.

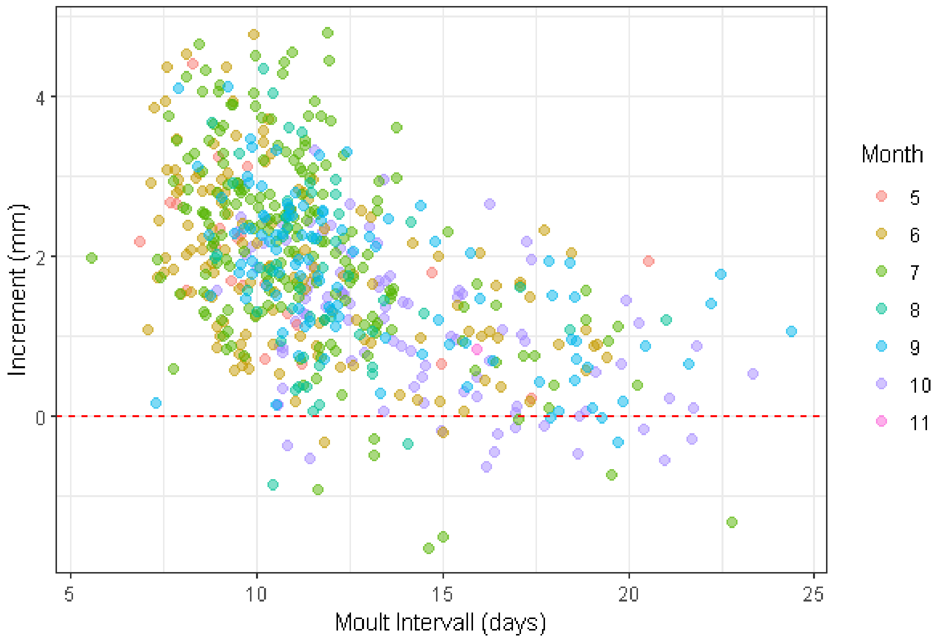

Figure 5-4*: Moult interval and corresponding moult increment of individuals between 21-30 mm over all sampled years (2018-2021), different colors resemble the months in which the individuals were taken.*

Short intervals at low growth increment were also observed only occasionally. Most of the data points on the top left of the plot, individuals that showed short moult intervals with large increments, originated from the month May, June and July. The animals of 21-30 mm size showed longer moult intervals in combination with smaller growth increments in the month September and October, indicating that their growth potential decreases towards autumn after peaking in June-July (Fig. 5-4).

Mortality

Mortality during the growth trials was calculated based on length class and after 30 days. While in the length classes with larger animals, up to 100% of the stocked animals died in 30 days, mortality in the length classes 21-30 and 31-40 mm, where the consistently largest sample sizes were obtained, ranged from 0 to 58%. Here an increase in mortality was observed for attempts later in the season compared to attempts earlier in the season. While the overall mortality in the trials with animals between 21-30 mm length between April and August was 9.1 ± 7.4% and did not exceed a value of 20%, 33.3 ± 15.5% died on average in the same length class between September and November. A similar picture, with 10.1 ± 10% mortality between April and August and 29.8 ± 9.6% mortality between September and November was found for the length class 31 ± 40 mm.

Dry weight condition Index (DWCI) and potential Increment

Besides strong annual differences in dry weight condition, e.g. between the years 2006 with generally very poor condition and 2010 with significantly better condition, inter-annual differences could also be observed.

***Table 5-1**: percentages of starving individuals in the length classes 21-30 mm 31-40 mm and 41-50 mm over month of the three illustrative years 2006, 2007 and 2010*

	2006 Length class (mm)			2007 Length class (mm)			2010 Length class (mm)		
Month	21-30	31-40	41-50	21-30	31-40	41-50	21-30	31-40	41-50
4	40.1	50.6	53.8	72.9	79.4	54.5	30.6	8.4	0
5	50.9	32.5	7.8	6.1	10.9	17.4	19.7	8.4	0
6	10.1	0	0	35.4	38.7	23.5	5.7	0	0
7				32.3	14.6	0	44.3	21.6	1.9
8	53.5	43	28.6	2.4	0	0	14.9	8	0
9	84.6	89.4	40.8	0	9.1	21.4	0	0	0
10	46.5	47.6	30.4	0	25.6	16.5	2.3	0.3	0
11	23.9	90.4	52.2	51.6	53.5	25	113.2	57.8	0.9
12	79.2	36.5	12.9	0	21.8	40			

Here, it was primarily the summer months that stood out for their significantly better condition. While the mean DWCI in April was still below 1 in all length classes and almost all years, the condition between May and October was significantly higher (Fig 5-5 A). In the years 2007 and 2010, the two smallest length classes best illustrate the inter-annual differences in condition. Similar to a bell, the mean conditions rise from a value around 0.87 in April to a value well above 1 in August, and then falls back to a value below 1 in the approaching winter. When comparing the different length classes, the two smaller length classes show higher DWCI values in summer compared to individuals of 41-50 mm length, in which less variation in DWCI could be observed throughout the season. In general, the animals sampled appear to be in comparable condition regardless of their size. In addition to the representation of the DWCI, a proportion of supposedly starving animals was calculated based on a sex-specific DWCI value of 1 (dashed red line in figure 5-5 A). It is noticeable that in the months in which the largest mean DWCI were measured, the fewest starving animals were observed. These were primarily the summer months from June to September. The lowest percentages of starving animals were observed in 2010 (Table 5-1).

Building upon this, for each month, length class and year, a potential increment was calculated for each individual that was above the minimum condition formulated by Sharawy *et al.* (2019). After sorting out all animals that could not have moulted due to their dry weight condition, and years in which a high data density was present are analyzed, seasonal differences in growth potential become visible (2010, Fig 5-5 C). In 2010 as well as 2007 a peak in potential increment can be traced, similarly to the observation in actual increment. Like the observations in the dry weight condition index, differences could be observed between potential increments of the summer months (June- October) and those of the winter months. The animals of the lengths 21-30 mm showed their best average potential increments in the month of June with about 2 mm. Slowly descending towards summer, lowest potential increments for this length-class were calculated for the month October. With increasing animal size, the largest potential increment predications shifted towards later season, with animals of 51-70 mm length showing their largest potential increments of more than 10 mm in September and October. Although seasonal patterns are discernible, they again differ greatly between the individual years observed. In 2007, for example, there is a clear trend toward larger increments in August and September, regardless of which length class is considered. In 2006, September appears to be significantly worse than other months in all length classes.

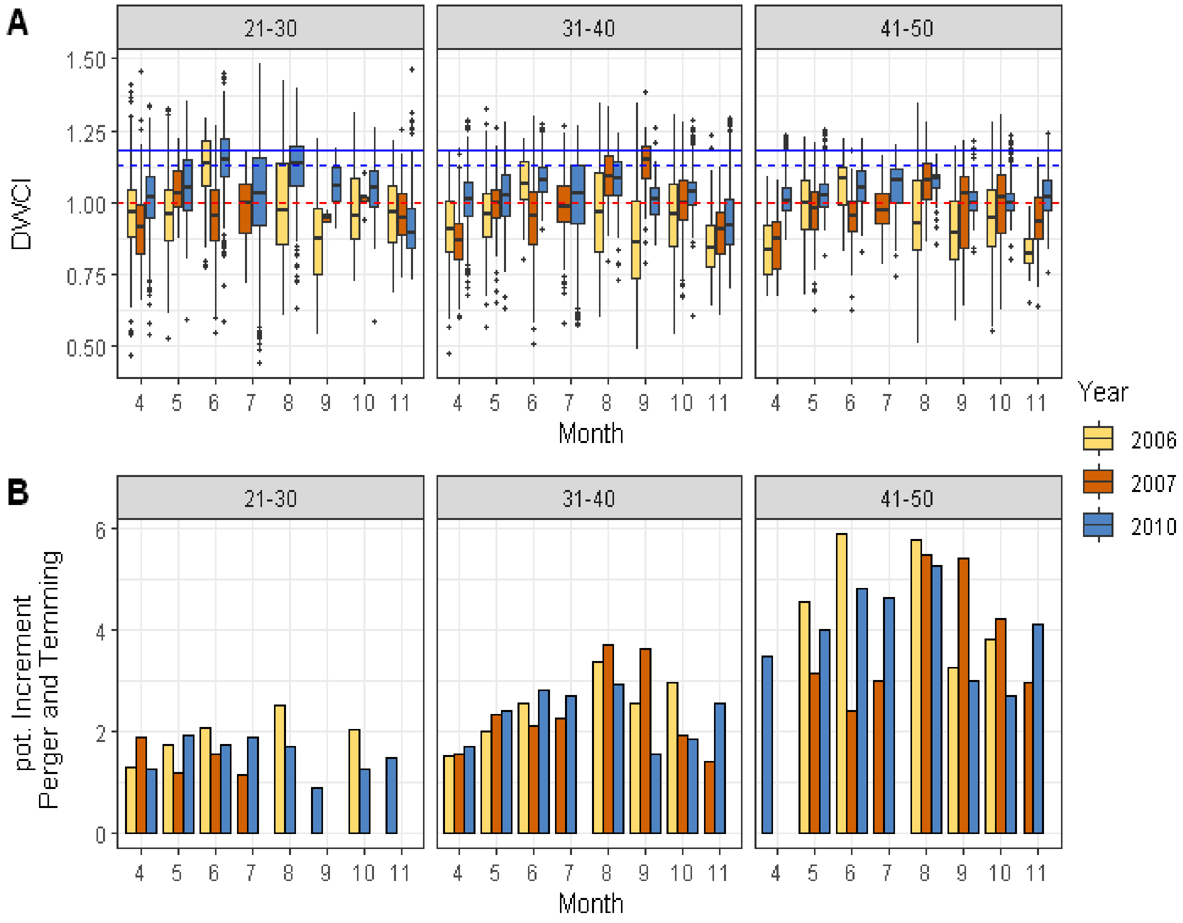

***Figure 5-5**: Dry weight condition index over months of the years 2006, 2007, 2010. Red line resembles a post moult condition, DWCI values below can be considered starving. The blue line represents the lowest DWCI for males (dashed) and females (continuous) to be considered in the potential increment calculation. B) Potential increments for individuals above the blue line in Plot A*

4. Discussion

Inter-annual growth patterns

The observations made in the present study suggest that winter egg recruits of the common brown shrimp, reaching the coast in late May and early June with a size of about 20 mm (Temming & Damm, 2002), are consistently responsible for the highest growth rates, and lowest mortality rates, observed in all length classes and throughout the season. Individuals of the same size, sampled at an earlier or later time in season, presumably summer egg recruits, showed poorer growth rates. These findings coincide with findings of Hufnagl *et al.* (2011a), where presumably overwintering summer egg recruits, sampled in early May, grew significantly worse than freshly incoming winter egg recruits of the same size, sampled a few weeks later. The authors attributed this to the age as well as the food histories of the two different cohorts. Based on the findings of a previous study on recruitment of the common brown shrimp (Temming & Damm, 2002), it was assumed that the animals captured in May were about 5 months older than the animals of the same size sampled in July. Larval release of winter egg recruits of the common brown shrimp is believed to occur in March to the beginning of April (Temming *et al.*, 2017). After a planktonic phase the freshly settled juvenile brown shrimp reach the coast at a length between 15 and 20 mm by the end of May (Temming & Damm, 2002; Temming *et al.*, 2017). Individuals larger 20 mm sampled way before that recruitment wave, can be considered older and hence no winter egg recruits. The results of the present study reveal, that not only overwintering summer egg recruits showed poorer growth potential, but also that summer egg recruits sampled in the same summer were likely to grow worse under the same conditions than winter-egg recruits. This effect was observed in all length classes investigated, and both when comparing moult increment and moult interval, so that the "well growing" cohort could be tracked through the season due to its significantly better growth performance. The growth rates being largest in length classes 21-30 and 31-40 mm in June and July, decreasing when observing later season in the same length class or larger length classes are in agreement with latest literate findings on growth (Perger & Temming, 2012; Sharawy *et al.*, 2019). Sharawy *et al.* (2019) observed the highest growth rates of 0.31 to 0.34 mm per day (females/males respectively) in individuals of 20-40 mm size in June and July. Both later in season as well is in larger individuals, growth rates were considerably poorer with 0.1 mm per day. According to Hufnagl and Temming 2011b, an individual of 25 mm length would show a growth rate of 0.28 mm per day at 17°C, and one of 35 mm length 0.23 mm per day. In the present study, comparable growth rates were observed in animals of the same size only in the months of June and July. Although some individual animals grew up to 0.57 mm per day, and such growth rates were observed in both Sharawy et al 2019 and Hufnagl and Temming 2011 b, these appear to be the exception. Probably the most exciting observation in the present study, made possible only by recording of individual moulting intervals, is that the animals can also improve their growth rate, not only by showing larger growth increments, but by reducing the timespan between two successive moults.

Food availability and starvation during winter

The common brown shrimp is an omnivorous species, preying on a variety of different benthic and epibenthic species. Through ontogeny, the preferred food changes from micro-plankton during the larval phase, over microorganisms such as larval stages of various invertebrates and bottom-dwelling copepods in the juvenile phase up to fish and benthic fauna such as polychaetes or mussels (Pihl & Rosenberg, 1984a; b; Van Der Veer & Bergman, 1987; Cattrijsse *et al.*, 1997; Van Der Veer *et al.*, 1998). These various nutritional components are available in varying quantities, depending on the season and recruitment variability of the prey populations. One reason why the winter egg recruits grow particularly well, could be the timing of the occurrence of various prey organisms of the common brown shrimp within the season (Kuipers & Dapper, 1984). *Crangon* larvae hatching in March/April would most likely benefit from the spring plankton bloom occurring during the same month in the North Sea (Reid *et al.*, 1990). The phytoplankton bloom is followed by a bloom of calanoid- and haparcticoid copepods (Huys *et al.*, 1986; Halsband & Hirche, 2001), both of which are known to serve as food for juvenile common brown shrimp (Pihl & Rosenberg, 1984a; Pihl, 1985; Boddeke *et al.*, 1986).

As summer progresses, and from July onwards, shrimp with a size of already 30-40 mm are likely to benefit from larval and juvenile stages of a variety of other benthic species, like gobies, nematodes and polychaetes (Gerlach & Schrage, 1969; Fonds, 1973; Bartels-Hardege & Zeeck, 1990). The new recruits (15-20mm) that continue to emerge in summer could also become potential food for the already 40 mm large winter egg recruits. Cannibalism of smaller conspecifics has been demonstrated in common brown shrimp and could thus be mentioned as a further reason why the food situation for winter egg recruits could be better compared to individuals that hatched in summer. Thus, the match-mismatch theory seems to be applicable not only to larval animals, but also to later life stages. In contrast, hibernating summer egg recruits are likely to experience longer periods of low food availability during the winter months. This phase can then lead to a non-genetic irreversible adaptation to poor food availability (Kinne, 1962). This adaptation is also supposed to be responsible for the growth differences observed by Hufnagl and Temming (2011 a). Nevertheless, the poorer growth potential of summer egg recruits within the same season they hatched (no hibernation) remains to be discussed. A possible explanation for this could be an evolutionary advantage for slow growing summer egg recruits. In general, fast growth, especially for species like *C. crangon* that is preyed on by various other species in the ecosystem is seen as advantageous. By growing faster than co-specifics, the escape from a size specific prey window is faster. On the other hand, it has been proven that in winter the food supply is insufficient for a large part of the shrimp population. Experiencing a phase of poor food availability at a smaller size would allow for a much higher survival probability and thus an evolutionary advantage compared to fast growing individuals.

Annual Differences

Another important finding that emerges from the results of the present study is that the observed seasonal growth behavior, the cohort effect, is significantly stronger in some years than in others. In 2019 for example, no significant growth differences were observed between animals of the same size, sampled in different months of the year. In 2018 and 2020, however, supposedly winter egg recruits outperformed summer egg recruits. Two different causes for this observation will be hypothesized in the following.

On the one hand, it is to be expected that depending on external influences, such as the larval drift or the mildness of the previous winter, the recruitment signals may vary. Egg development time in *C. crangon* is believed to be mainly influenced by water temperature. A cold spring would hence lead to a prolongation of the winter egg development. With increasing water temperatures, a sudden hatching of many winter eggs would result in a more synchronized appearance of recruits. Conversely, could a mild winter lead to early hatching recruits, and thus to a potential miss match scenario of the common brown shrimp larvae, with the spring plankton bloom, which is rather driven by day light duration than temperature. This theory would be supported by the fact that the months of February and March in the years 2018 and 2020 were significantly colder than those in 2019. The analysis of length frequency distribution in combination with density observations in spring 2019 revealed no clear recruitment signal. Density in 2019 on the sampling site in Büsum was unusually high, even in early spring, suggesting that unusual amounts of shrimp overwintered from 2018 to 2019. Thus, the winter egg-recruitment signal may have been drowned in this quantity of animals. Leading to growth observations on primarily overwintering summer egg recruits in spring 2019. Alternatively, the arriving winter egg recruits may have served as food for the overwintered co-specifics already sitting on the mud flats in large densities. Both cases would explain why no high growth rates, usual for winter egg recruits, were observed in 2019.

When comparing DWCI in one length class but throughout the season, only better conditions in summer and worse conditions in winter could be observed, but no clear signal indicating better conditions in winter egg recruits. Good conditions in summer and bad conditions in winter are consistent with the findings of other authors (Hufnagl *et al.*, 2010b). Generally, this can be attributed to the respective food supply. While in summer the strong primary and secondary production allows the high DWCI values, in winter food is scarcer and the condition of the animals decreases after a prolonged period of starvation (Hufnagl *et al.*, 2010b). However, based on the results of Perger and Temming 2012, it is actually assumed that growth and DWCI are strongly linked. Accordingly, in a phase in which one length class showed significantly better growth, the conditions should also be higher than in other phases. When considering the potential increment, however, the expected effects become visible. Here, only animals that would have formed a positive increment based on their dry weight condition are included in the calculation. For the description of the differences that can be explained by the cohort effect, the selection of the animals to be considered is of crucial importance. Especially in cases where a pre-selection is made, as for example in the growth results of animals with a length of 21-30 mm (here only increments were taken into account that occurred maximum 5 days after capture), it must be considered that thereby possibly starving animals are excluded from the results. Although the potential increment seems the better way to discover seasonal patterns, induced by a cohort effect, there seems to be an overestimation of increment especially for larger individuals. Compared to literate values and the finding during this study, increments of 6 mm and above, seem highly unlikely. Nevertheless, and especially since the error is the same for all animals of this size, the potential increment is useful for in-season comparisons, but one should not assume a potential growth rate with these values. The presentation of the percentage of starving animals in the season makes it seem as if there are less starving animals in the winter egg cohort. In the patches where summer egg and winter egg recruits are mixed in one length class, this leads to a larger scatter in both dry weight data and growth results.

Conclusion

The results of the present study have shown that the cohort effect exists, and that its expression can vary in strength from year to year. Factors, such as winter mildness, the stock development of the previous season and early life stage survival of the shrimp seem to determine, how strong the signal of the cohort effect is. The previously undescribed growth differences in animals of the same size caught at different points in the season also have an impact on model approaches, which to date rely on reliable growth data. The results of the present study suggest that to maintain the productivity of the shrimp stock, a protection especially of the winter egg cohort may be of particular importance. Previous studies suggest that most animals caught in the main fishing season in autumn must originate from winter egg recruits (Hufnagl & Temming, 2011b). Further, there are signs that indicate a recruitment overfishing in winter months. Higher fishing pressure of the coast of the Netherlands, lead to significantly reduced catches in the following summer/ autumn season of north Frisian coast (Respondek *et al.*, 2022). This, combined with the knowledge that these animals also grow significantly better compared to later spawned animals, could make the protection of these cohorts beneficial to both the fishery and the stock. Protection of the winter egg cohort can be achieved both by protecting the adult stock in winter, for example by a fishing-stop in a period when females are bearing eggs, or by measures that conserve growing winter egg recruits before they reach a marketable size. Efforts towards the conservation of juvenile shrimp, due to a mesh size increase have shown that survival probability of smaller Individuals increases. Since still a lot of undersized shrimp are caught, a further increase in cod-end mesh size could help in sparing the small winter egg recruits during summer. The results of Respondek et al (2022) have shown that measures can also be regionally effective. Accordingly, the well-growing cohort should only be spared where it is expected to be at the respective point in the season.

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Manuscript 4: Establishing a method for in situ determination of the moult interval and moult stage from frozen field samples of shrimp. A case study on the common brown shrimp (*Crangon crangon)*.

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Growth, *C.crangon*, cohort, seasonality, winter eggs, summer eggs

Abstract

The frequency at which two successive moults occur in shrimps, plays a decisive role for the determination of growth rates being composed of moult increments and moult intervals. The experimental determination of moult intervals requires great experimental effort, and the results are strongly influenced by feeding conditions. In the present study, we describe a method to calculate the moult interval for groups of individuals from field samples. Based on the stress strain analysis, known from material science, we have developed a device, which allows the empirical determination of the carapace hardness in C. crangon. During calibration experiments carapace hardness in different length classes was related with time after the observed moult. After calibration, in five field samples from 2021 freshly moulted individuals were identified via carapace hardness and their proportion in the total catch allowed a length specific calculation of the moult interval. The study lays a methodological foundation for moult interval determination in other, small rapidly moulting Crustacean species.

1. Introduction

The importance of reliable growth data in commercially exploited crustacean species is demonstrated by the ever-failing attempts of age determination (Hartnoll, 2001; Kilada & Driscoll, 2017), and hence the wide variety of non-age-based management approaches (Penn *et al.*, 2019). In species such as the common brown shrimp (*C. crangon*), where biological characteristics like an extended spawning season and a short lifespan do not allow for the assignment to a traceable cohort, growth estimates are based mainly on laboratory experiments (Hufnagl and Temming 2011a, b; Temming *et al.*, 2017). In many growth studies, groups of shrimp with the same starting length were simply fed ad libitum over a longer period and the overall growth rate in mm/d was subsequently extracted (Hufnagl and Temming 2011b). However, only few studies (Oh & Hartnoll, 2000; Schatte & Saborowski, 2006; Perger & Temming, 2012) have documented how the individual growth rates are composed in terms of moult interval and moult increment.

While the growth increments in the common brown shrimp have been widely studied and are, due to their comparative ease of measurement, supported by a larger body of data, observations on the individual moulting intervals are rather rare (Hufnagl & Temming, 2011b). To record individual moult intervals, animals must be observed over a period of least two consecutive moults. The dilemma that usually arises here is that during this time the animal is significantly influenced by the housing conditions in the laboratory, which makes the subsequent evaluation of the results error prone to the housing conditions (Delbare *et al.*, 2015). In many of the studies compiled by Hufnagl and Temming (2011b), the species was found to be difficult to maintain in the laboratory and observations of little to no growth were rather common.

In methods such as the instantaneous growth rate (IGR), difficulties during husbandry are circumvented by observing only moults of animals over a short period after their catch. Then the proportion of moults that have occurred in relation to individuals that have not moulted is used to calculate an interval for a group of animals (Quetin, L. B., Ross, R. M., & Clarke, 1994; Hart, 2001; Perger & Temming, 2012; Sharawy *et al.*, 2019). Studies on other crustaceans however, give reason to be concerned that moults occurring shortly after capture might be strongly related to the previous capture and handling procedures (Ferraris *et al.*, 1987; Coyle *et* *al.*, 2010). The occurrence of these so-called stress moults increases the risk of overestimating the growth of the animals when using the IGR method.

Moulting interval in common brown shrimp was primarily believed to be a function of size and temperature (Hufnagl & Temming, 2011a). However recent studies indicated that other factors, such as the availability of food, also have a significant influence on frequency at which an individual can moult (Oh & Hartnoll, 2000; Saathoff *et al. unpublished*, 2022). Reports from shrimp fisherman in the North Sea, suggest that further factors, such as water turbidity after storm events, could influence the moulting behavior of *C. crangon*, as they observe a more frequent appearance of “soft” shrimp after storm surges. Although the moulting interval plays a crucial role in the growth rate calculation of the species, the factors that influence it are still not fully understood. This, together with the problems of determining the interval in the laboratory, highlights the importance of a method for determining moulting interval from frozen field samples.

Hence, the objective of the present study was to establish a method which enables the prediction of in situ moulting intervals for groups of individuals (5 mm Length classes), based on the changing exoskeleton properties during the moulting cycle. We devolved a device which enables an empirical hardness measurement of an individual’s carapace, following the stress strain analysis, commonly used in material science. We further described characteristics in common brown shrimp, which in combination can be used to identify freshly moulted individuals within a sample. Using the number of freshly moulted animals, we enabled the calculation of a moulting interval, and tested the new method on frozen field samples and present the method as a possible tool for use on other crustacean species.

2. Material and Methods

Sampling

Animals were sampled in Büsum (54°07 N, 08° 51 E) during low tide, using a push net at about one-meter water depth. The live animals were then transferred to the Institute for marine Ecosystem and Fisheries Science in Hamburg in ventilated 30-liter containers filled with artificial seawater, which was adjusted to in field salinity and temperature prior to the transport. Depending on their later use, the animals were treated differently from there on. Samples for the validation of the method and for determination of the in-field moult rate were frozen separately at-80°C immediately after the arrival in Hamburg, and 1-2 hours after their catch. Individuals taken for moult experiments were held in an acclimatization tank prior to their stocking in the experiment.

Shrimp with known moult stage for validation

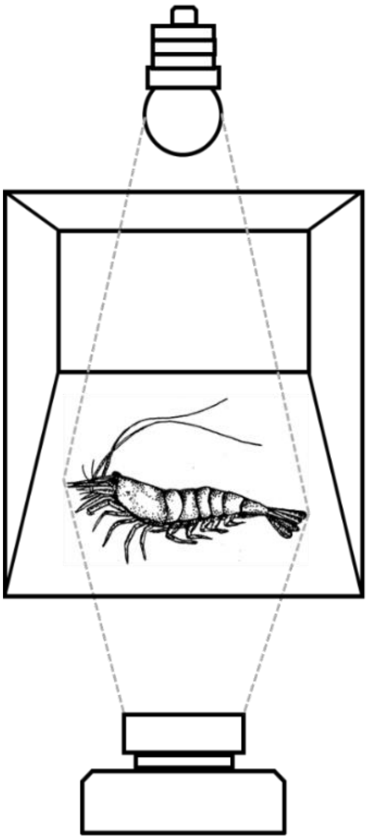

Figure 6-1: Schematic illustration of the experimental setup for determining the exact moment of moult.

To know the exact moment an individual moulted, and thereby the beginning of the integuments hardening, a permanent monitoring of the individuals during both day and night was indispensable. After an acclimatization period of around 24 h, the animals were kept individually, in a tank specially designed for their observation (17°C 12/12h light regime). For this purpose, 48 individual compartments were combined to form a table, of which the Plexiglas bottom allowed an observation of the animals using a camera and transmitted light (Fig. 6-1). Two Infrared (IR) diode strips provided enough background light to detect the animal’s movement and the exact moment of moult, even at complete darkness. The frame rate of the observing cameras was set to 3 pictures per minute, so the moult of the individuals was accurately determinable to 20 seconds. Animals were fed once a day, while feces and left-over food was removed from the individual compartments. After an individual had moulted, both the animal and its exuvia were removed from the tank and the animal was frozen at -40°C until further processing. The time between moulting and removal of the animal defined the curing time of the new exoskeleton for the respective individual. By repeating this procedure, animals were collected whose moult occurred between 0 and 48 hours prior to their removal. In a further setup, where the individual´s newly formed exoskeleton cured for more than 48 hours, animals were also kept separately (17°C 12/12h light regime) and were fed once a day, but the moment an individual moulted was only determined to an accuracy of 8 hours. In this way, animals were collected whose last moult occurred between 50 and 300 hours prior to their removal.

Field samples

For the application of the method on field samples, animals were transferred to the institute for marine ecosystem and fishery sciences in Hamburg on five occasions in 2021. Since the field prevailing temperature is known to be an important driver of the animals' moulting interval, samples were taken in months with widely varying water temperatures in 2021, Salinity and water temperature were monitored during sampling using a pre-calibrated salinometer (HI98319, Hanna Instruments).

Hardness measuring apparatus

To determine the hardness of the exoskeleton, a device similar to a texture analyzer used in the food industry was developed. Based on the principle of a stress strain analysis in material science, measuring applied force while the lowering of an indenter, in our case a 0.45 x 23 mm surgical needle, through the carapace of the animal served to determine the hardness. Therefore, the surgical needle was mounted to a 100 g loading cell, which in turn was mounted onto an adjustment aid. The measuring unit was mounted on a threaded rod, with the help of which a 5-volt electric stepping motor could move the indenter up and down in 10 µm steps (Fig. 6-2 A). Using a microcontroller (Arduino Nano) the motor operation was programmed, to lower the surgical needle until the load cell registered an increase in force. The force increase, triggered by reaching the carapace, initiated the actual measurement process, in which the surgical needle penetrated the carapace in 10 µm steps over a total distance of 2 mm. The force registered by the load cell after each 10 µm step was read via a serial monitor. For the later analysis, two different measurements were taken whilst puncturing the carapace. First the, the applied force at rupture, referred to as yield strength, as well as the force increase from the beginning of the measurement until reaching the yield strength, referred to as slope, were extracted for each individual.

The actual hardness measurement

Both individuals from the field and ones with known moult stage were thawed and blotted dry on tissue paper. After determining the individuals' length and wet weight, the animal was then placed under the measuring apparatus so that the carapace, as the target of the surgical needle, pointed upwards. The measuring head was aligned so that the surgical needle could be lowered centrally onto the carapace (Fig. 6-2 B). The measuring program was then started, and the data measured by the loading cell was transferred to a data sheet. After completion of the measurement individual dry weight was determined after 24 h of freeze-drying.

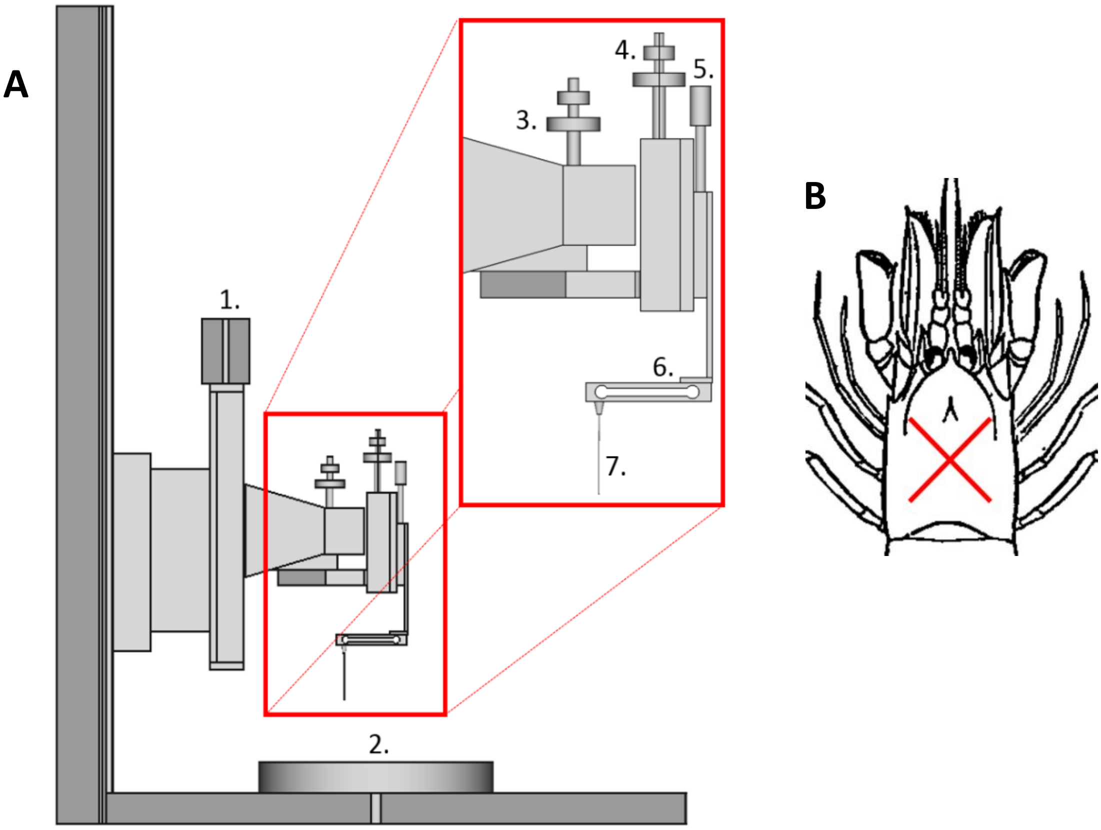

Figure 6-2: *A) schematic illustration of the hardness measuring device. 1. Electric motor that moved the measuring head in z direction. 2. Solid base on which the sample is placed. Precision mechanics to vary the position of the measuring head in- y direction (3.), x direction (4.) and z direction (5.). 6. 100 g loading cell to detect the force applied when measuring. 7. Surgical needle as a measuring probe. B) Drawing of the position where the surgical needle should pierce the carapace approx.*

Length, weight, dry weight, sex, and dry weight condition index (DWCI)

Length was measured as total length (TL1) to the nearest millimeter from the tip of the uropodes to the tip of the scaphocerite using graph paper. Wet weight was determined by blotting the individuals dry on paper tissue (scale: Satorius ± 0.001g). Dry weight (DW) was determined after 24 h freeze drying (scale: Satorius ± 0.0001g). Sex was determined from the endopodites of the first and second pair of pleopods and by the presence or absence of an appendix masculina using a binocular. To minimize handling stress, sex was not determined prior to the calibration experiments, but after the end of the experiment, on thawed animals.

Dry weight condition index and water content were calculated as:

$$DWCI = \frac{DW}{a \cdot TL^{b}}$$

and

$$Water\ content = \frac{DW}{W} \cdot 100$$

With DW= dry weight, TL = total length and a = 0.00199/0.00394 and b = 2.92/2.72 (Sharawy *et al.* 2019) for females and males (f/m, respectively).

3. Results

Moulting

During 18 month of observations a total of 1956 moults were documented of which 81 percent occurred during nighttime, preferable 0-2 hours after the artificial sunset. Although the individual's activity level was reduced significantly pre- and post moult, the actual moult event, beginning with the rupture of the pleural suture to the complete removal of the old exuvia, took about a minute, so that at a frame rate of 3 pictures a minute, mostly 4 pictures were needed to identify the exact moment of moult (Fig. 6-3 A-D). Generally, a quite homogenous distribution of moults could be observed during an experimental run (Stocking to the moment the last individual moulted), with the occurrence of 2-4 moults daily at a total of 48 individuals and a water temperature of 17 °C. With decreasing numbers of individuals towards the end of an experimental run, the number of moults also decreased. However, isolated events in which up to one third of all stocked individuals moulted within the same night were observed (Table

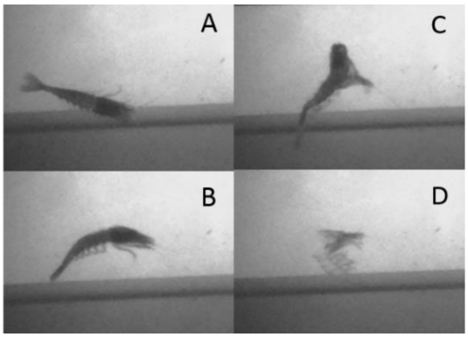

Figure 6-3*: moult event, observed via transmitted IR light. Exactly 80 seconds passed*

6-1). While in two cases, this was observed immediately after stocking, in another trial, these mass moulting events occurred up to 8 days after stocking.

Table 6-1: *Moult distribution of three exemplary trials. Run A: from April 2020 with a comparatively homogeneous distribution of moults until day 20, Run B: from December 2019 with a mass moulting 8 days into the experiment and Run c: from May 2020 with mass moulting right at the beginning of the run*

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
N moults run A	4	2	3	3	2	1	2	2	3	0	1	2	2	3	1	0	0	2	1	2
N moults run B	2	3	1	2	2	3	1	13	1	0	3	2	1	2	0	2	2	1	2	1
N moults run C	9	7	0	1	2	1	3	1	1	2	0	2	1	0	0	2	1	1	1	1

Hardness measurement

A measurement path for determining the carapace hardness can be described in two phases. In phase one, the measuring (surgical needle) head is continuously lowered onto the carapace, and the loading cell detects a constant force increase over the traveled distance of the measuring head (slope in Fig. 6-4). This continues until the carapace finally gives way and the surgical needle pierces through (yield strength in Fig. 6-4). The second phase is characterized by a rapid decrease in measured force. After rupture, the intender penetrates further into the sample and trough softer material. This phase is characterized by rapidly changing force measurements of the loading cell. Figure 6-4 shows the hardness measurement of two

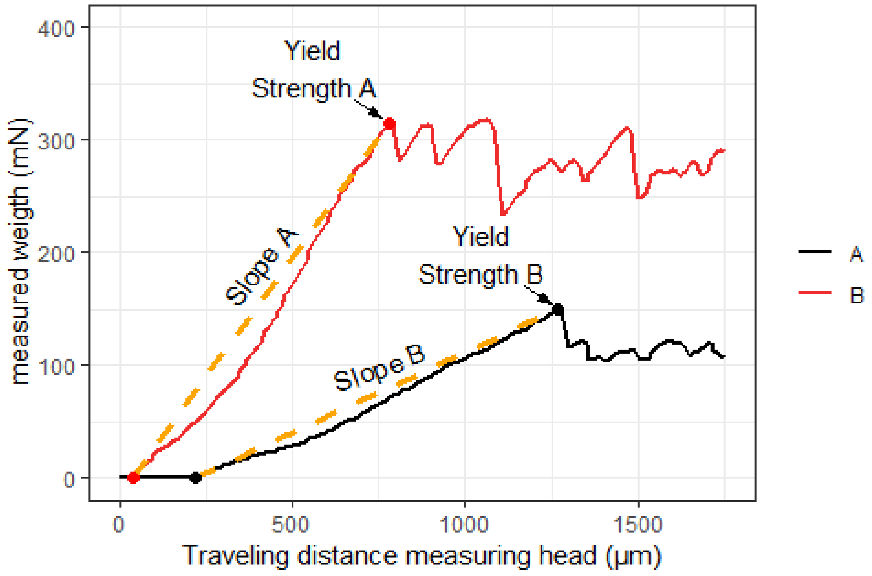

Figure 6-4: *Illustration of two measurement paths in animals of the same size (35 mm) but with different curing times after moulting. Individual A cured for approx. 5 days, while individual B cured for 12 hours.*

individuals of the same size, which differed in their curing time. The carapace of the individual with the longer curing time (individual A) withstood a significantly higher force than the animal of the same length, which had less time to cure.

Besides an improved yield strength, the traveling distance of the measuring head, to reach the yield strength differed largely between the individuals. The calculated slope from measuring distance and applied force was significantly higher in the individuals with a longer curing time, compared to the softer less cured individual. Hence two different variables were found to be useful in the determination of the carapace hardness. Both the force needed to penetrate the carapace (Yield strength) and force applied over a given measuring distance (Slope) increased significantly with curing time and size of the individuals.

Yield strength in reared shrimp

Yield strength in shrimp held for calibration of the method, varied significantly with individual size (Linear regression model $p<0.001$, Tab. 6-2) and the time between moult and extraction of the individual (Log-Linear regression model $p<0.001$ Tab. 6-2). Both the individual’s size as well as curing time had a positive effect on the hardness reading. The force needed to rupture the carapace varied between 20 and 80 mN in soft individuals of 21-25 mm size, to over 600 mN in fully cured individuals larger 60 mm (Fig. 6-5). Especially immediately after moult, but up to a curing time of around 48 h, yield strength was significantly lower, compared to readings in individuals that cured for 50 h or longer. From a comparable low reading in all length classes, a gradual increase in yield strength could be observed with curing time. A stronger increase in yield strength was observed with increasing animal size (Fig. 6-5 B). While in length class 21-25 mm highest readings were around 150 mN in length class 55-60 mm a curing time of 10+ hours resulted in a yield strength reading of >400 mN. The early hours after moult in which the recent moult was successfully detectable via yield strength reading was narrowed down to a range between 0 and approx. 30 hours after moulting (orange dots in Fig 6-5 B).

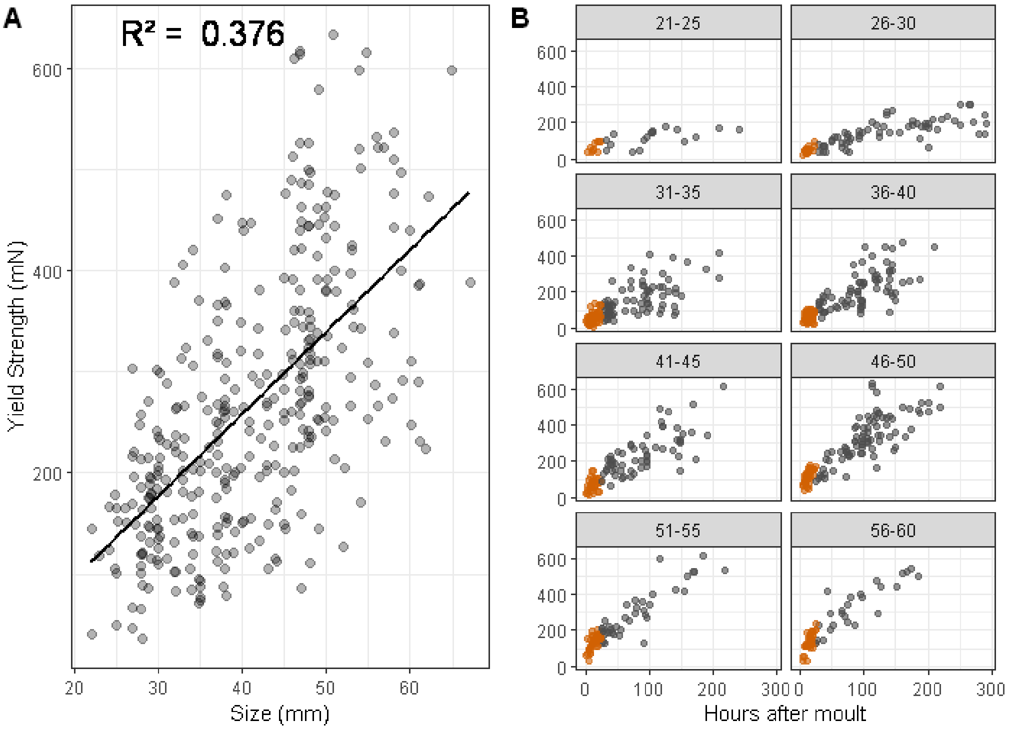

Figure 6-5: *Yield strength of individuals from the lab that cured for more than 50 hours (A). Yield strength over curing time of the exoskeleton for different size classes (B). Orange dots in plot B indicate individuals that were extracted up to 24 hours after their moult, and that could thus be considered freshly moulted*

Slope

Slope for all measuring paths was determined from the first contact of the intender with the sample to a traveling distance of 500 µm. Slope in for calibration reared shrimp varied significantly with curing time and size of the individuals, reaching highest values of 0.6 in individuals of 55 to 60 mm size and after 200 hours curing time (Linear regression model $p<0.001$, Tab. 6-2). Unlike to the results in yield strength, especially in the two smallest length classes, comparably low slope readings were detected in individuals with long curing time. However, from a size of 30 mm, the slope could also be used to identify the most recent moult with a high degree of certainty. The period of around 24 hours during which the soft exoskeleton produced a small slope reading is indicated by orange dots in figure in figure 6-6 B.

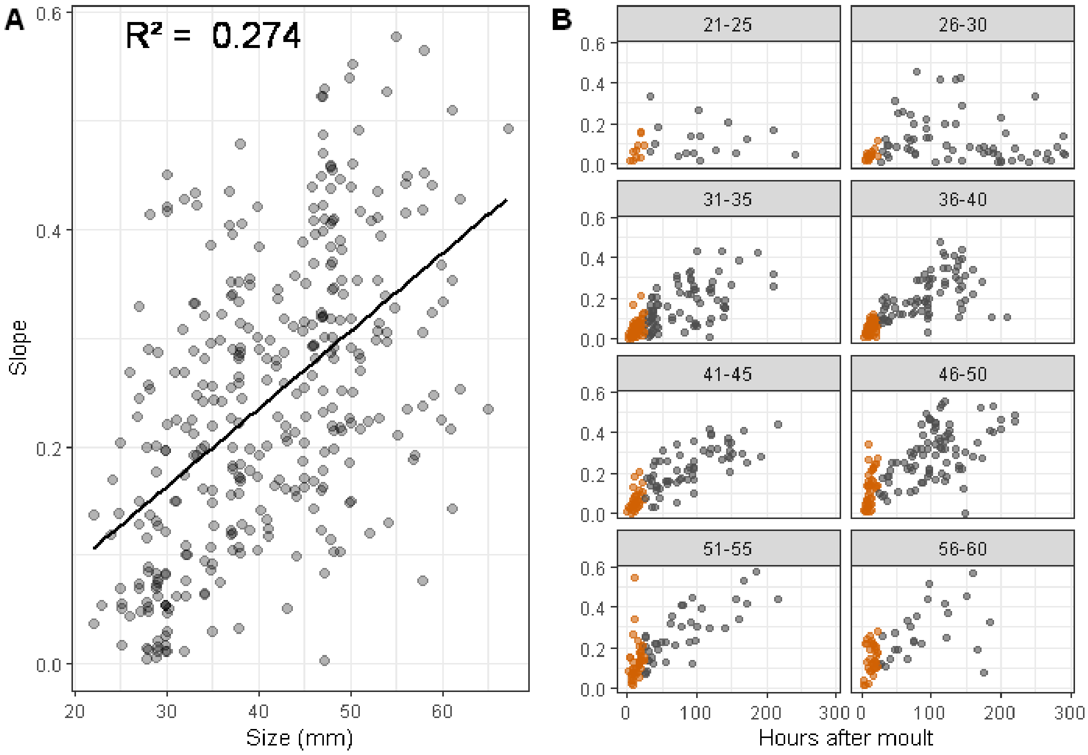

***Figure 6-6**: Slope of the measuring path of individuals from the lab that cured for more than 50 hours (A). Slope over curing time of the exoskeleton for different size classes (B). Orange dots in plot B indicate individuals that were extracted up to 24 hours after their moult, and that could thus be considered freshly moulted.*

Dry weight condition and water content

Dry weight condition index increased significantly with the time between extraction of the individual and the recent moult (Linear regression Model $p < 0.001$, Tab. 6-2). In the first 48 hours after moult, DWCI ranged in all individuals between values of 0.8 and 1.2, increasing steadily from there on to values of up to 1.6. Similarly the water content was highest in individuals immediately after moult and decreased gradually with curing time. Water contents of over 80% immediately after moulting decreased steadily to values of less than 70% with increasing curing time.

In addition to the relationship with curing time, a linear relationship was found between water content/DWCI and the two hardness proxies, yield strength and slope (Tab. 6-2). Both in field samples (green data points in Fig. 6-7) and in animals kept for calibration (grey = cured for more than 24 h, orange = cured for less than 24 h, Fig. 6-7), low yield strength and slope values were observed at high water content and vice versa (Fig 6-7). In some cases and in individuals observed during laboratory experiments, water contents that were comparable to freshly moulted individuals were found in animals that cured for more than 100 hours. In particular, animals that had been in the laboratory for a longer period than their usual moulting interval, showed high water contents and low DWCI when removed and subsequently analyzed.

Table 6-2*: All Regressions*

Dependent variable	Predictor	Coefficient a	Coefficient b	significance	R²	F value
Yield Strength	length (>50 hours after moult)	8.132	-66.628	***	0.376	208.1
Slope	length (> 50 hours after moult)	0.007	-0.051	***	0.274	131.5
Ln(Yield Strength)	Ln(hours after moult)			***		
Ln(Slope)	Ln(hours after moult)			***		
Water content	Yield Strength (26-30 mm)	-0.020	79.004	***	0.483	146.7
Water content	Yield Strength (31-35 mm)	-0.017	79.680	***	0.553	875.5
Water content	Yield Strength (36-40 mm)	-0.014	79.900	***	0.666	953.9
Water content	Yield Strength (41-45 mm)	-0.012	79.899	***	0.668	570.2
DWCI	Yield Strength (26-30 mm)	0.0016	0.890	***	0.582	218.3
DWCI	Yield Strength (31-35 mm)	0.00125	0.915	***	0.531	802
DWCI	Yield Strength (36-40 mm)	0.00093	0.916	***	0.586	675.2
DWCI	Yield Strength (41-45 mm)	0.00089	0.923	***	0.669	575
Water content	Slope (26-30 mm)	-8.062	77.219	***	0.196	39.5
Water content	Slope (31-35 mm)	-11.752	78.770	***	0.424	522.3
Water content	Slope (36-40 mm)	-10.866	78.620	***	0.391	309
Water content	Slope (41-45 mm)	-9.966	78-380	***	0.329	141.7
DWCI slope	Slope (26-30 mm)	0.756	1.025	***	0.297	67.8
DWCI slope	Slope (31-35 mm)	0.904	0.973	***	0.445	568.8
DWCI slope	Slope (36-40 mm)	0.720	1.001	***	0.342	250.2
DWCI slope	Slope (41-45 mm)	0.763	1.029	***	0.360	161.7

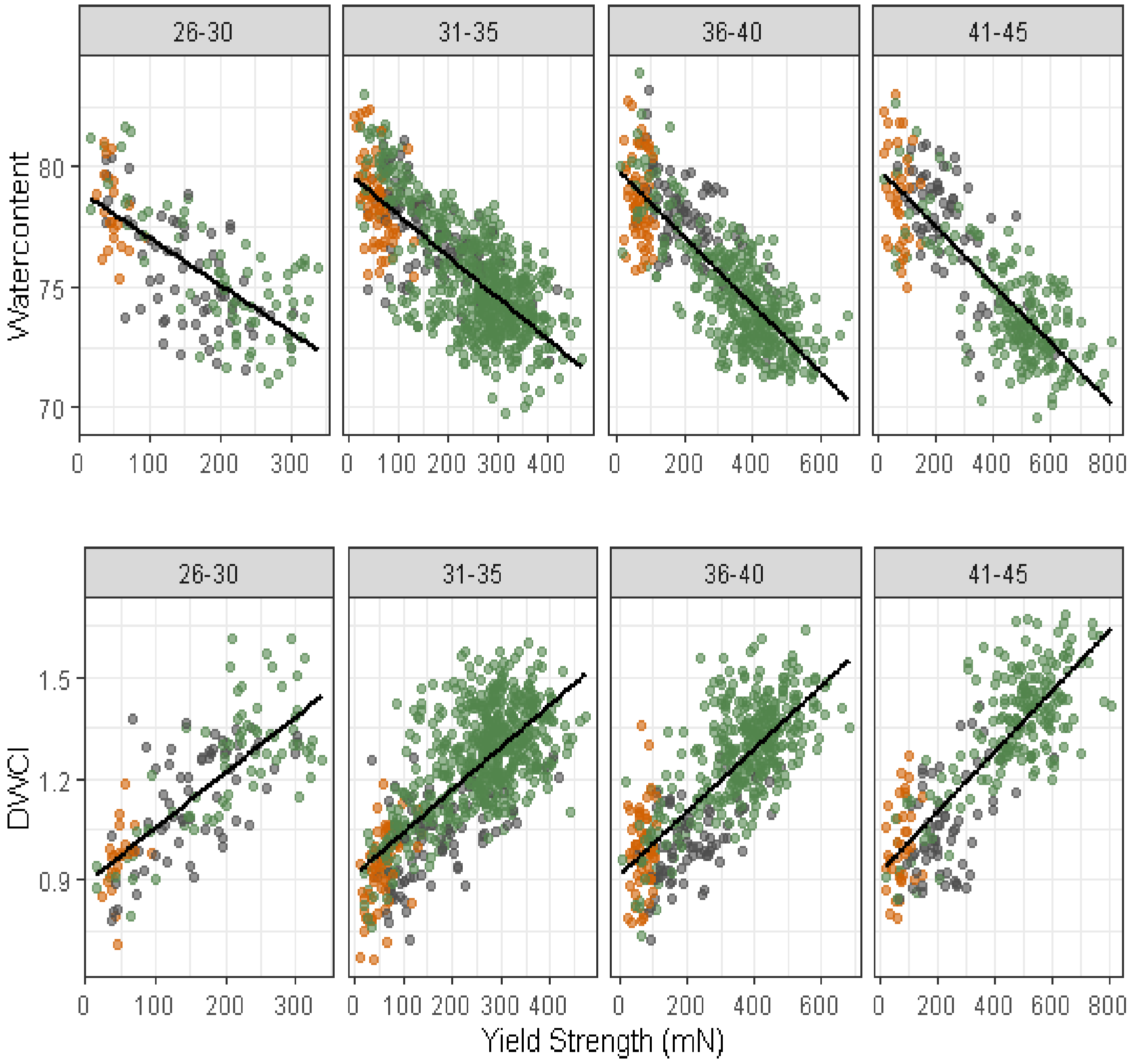

***Figure 6-7**: Exemplary representation of yield strength versus water content and DWCI for animals of lengths 26-45 mm, green shows field samples, grey reflects animals from the laboratory with a curing time of more than 24h, orange data points show animals with less than 24 curing time.*

Field Samples

***Table 6-3**: List of threshold values for the different length classes*

Length class	Water content	Yield strength	Slope
21-25 mm	75.2 %	98.1 mN	0.16
26-30 mm	75.4 %	95.8 mN	0.11
31-35 mm	75.4 %	136.8 mN	0.21
36-40 mm	75.8 %	128.9 mN	0.12
41-45 mm	75.0 %	144.7 mN	0.20
46-50 mm	74.6 %	177.8 mN	0.34
51-55 mm	74.6 %	204.7 mN	0.54
56-60 mm	75.1 %	237.4 mN	0.77

Based on the calibration experiments, a combination of three proxies were found to be valid in order to detect a recently (last 24h) occurred moult. The combination of a high water content, thus a low DWCI, with low carapace hardness, indicated by low readings in yield strength and slope, were characteristics only found in freshly moulted individuals. Figure 9 shows the water content, yield strength, and slope (size of data points) of individuals in each of the five samplings from 2021. Based on the set of thresholds (Tab. 6-3), orange data points were animals identified as moulted within the last 24 hours, whilst gray data points resemble animals in which their resent moult occurred more than 24 hours ago.

From the relative proportions of freshly moulted individuals to ones that cured for more than 24 hours a moult interval was determined for the respective length class and sampling month. Differences in moult interval were found both between different month and between different length classes in the same month. The intervals calculated in this way, as well as the water temperature on the day of capture and the respective sample size, are shown in Table 6-4. It is noticeable that in almost all months, except June, a shorter interval is calculated for the smaller animals. June and July, months with high water temperatures, had short intervals, whereas in October, when the water was colder, the intervals were longer. However, the sampling month Arpil with relatively short interval at low water temperature is somewhat out of line. Furthermore, it becomes apparent that low sample sizes do not allow for the determination of the moulting intervall. This is reflected in the observation for the month of August, in which apparently no freshly moulted animal was sampled in both length classes (n= 30).

***Table 6-4**: calculated moulting intervals based on the proportion on freshly moulted individuals within the catch for the 5 respective sampling month in 2021*

		Length class 31-35 mm		Length class 36-40 mm	
Month	Temperature	N	Length class 31-35 mm	N	Length class 36-40 mm
April	8.1 °C	140	6.6 days	65	11.2 days
June	17.3°C	128	11.6 days	38	9.5 days
July	18.4°C	198	8.6 days	34	12.3 days
August	18.3°C	29	-	30	-
October	9.7°C	103	20 days	208	34.6 days

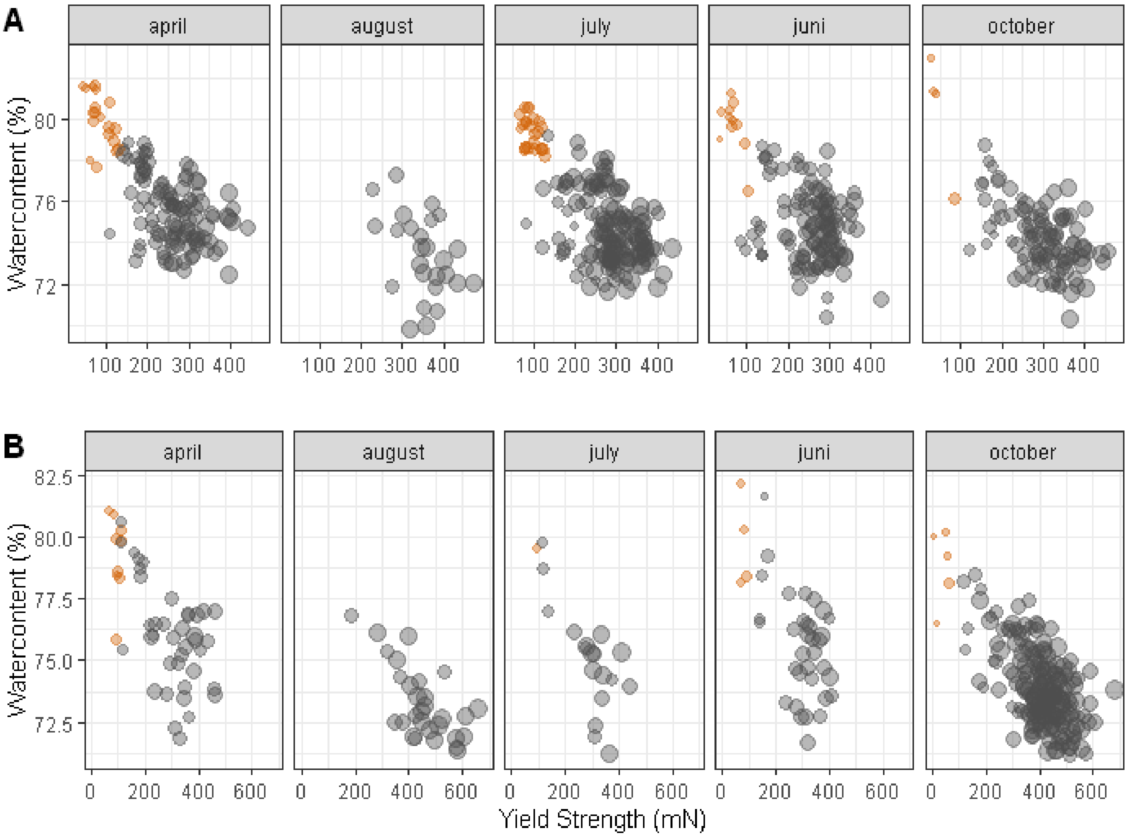

***Figure 6-8**: Water content over Yield strength and Slope (size of the data points) for individuals of 31-35 mm (A) and 36-40 mm (B) in five field samples of the year 2021. Animals classified as moulted in the last 24 hours based on laboratory results are shown in orange.*

4. Discussion

Due to the lack of moult- enduring hard structures, a year round lasting spawning period and a life span of about one year, age determination in common brown shrimp is merely impossible (Hartnoll, 2001). This is the reason that to date growth estimates for dynamic population models are mostly based on laboratory experiments (Temming *et al.*, 2017; Günther *et al.*, 2021). Recently progress was made with regard to the estimation of moult increments from field samples utilizing dry weight condition data (Perger & Temming, 2012; Sharawy *et al.*, 2019). What was missing so far, however, was a complementary approach to extract also the moult increment from field samples. The present study highlights, that properties of the exoskeleton and changes in body composition during the moulting cycle of common brown shrimp have the potential to close this gap. Like the dry weight condition method (Perger and Temming 2012) also the here presented method requires laboratory calibrations, but the subsequent application does not require live individuals and aquarium facilities. Rather, the share of freshly moulted individuals in relation to the number of not freshly moulted individuals can be used to calculate a moulting interval (Quetin & Ross, 1991), for a group of individuals.

Validation

During the validation trials 81% of the observed moults occurred during nighttime. This is in line with findings of other authors in other decapod crustacean species (Hess, 1941; Fowler *et al.*, 1971; Hagerman, 1976). It is assumed that the shrimps prefer the night, with the lower brightness and thus greater protection from predators, to perform an upcoming moult. Immediately after moulting, the animals are less mobile due to their soft exoskeleton and also less able to flee, which is why moulting in poor visibility conditions could be advantageous (Hartnoll, 2001). The distribution of observed moults over one experimental run was mostly homogeneous. Two to four moults at 48 individuals corresponds to a moulting interval of around 10-20 days, which at a mixture in size, a given temperature of 17°C and ad libitum feeding seems reasonable, indicating good husbandry conditions (Hufnagl & Temming, 2011b; Sharawy *et al.*, 2019).

However, several situations occurred in which up two one third of the held individuals moulted within one night. Especially at the beginning of an experiment, this can be attributed, to a so-called stress moult (Ferraris *et al.*, 1987; Coyle *et al.*, 2010). The animals, stressed due to their catch, transport and stocking, could perform a moult earlier than usual to repair possible damage of their exoskeleton. On one occasion, such a mass moulting event occurred several days after the animals were stocked. Reasons for this remain unexplained, but there is evidence for a non-homogenous distribution of moults in other crustaceans (Naylor, 1999; Franke & Hoerstgen-Schwark, 2013). Hence the homogeneity of moults in C. crangon and deviations thereof should be a focus of further studies, using the newly established method.

After the analysis of shrimp with known moult stage, three properties of both the body composition and carapace hardness were identified to be useful in the determination of recent moult events in frozen samples. Both yield strength, the actual force the carapace withstood while being punctured, and the force increase over the traveled distance of the indenter (slope) were found to increase significantly with the curing time of the newly formed exoskeleton. Both measurements in combination with DWCI and/or water content of an individual were used to predict a recently occurred moult. Why the three measurements are noticeably different in freshly moulted animals than in animals that are, for example, about to moult, can be understood by looking at the different moult stages within the crustacean moult cycle.

The different stages of the crustacean moulting cycle were first described by Drach (1939). During Stage A, the phase immediately following the breaking of the ecdysal sutures and the shedding of the old exoskeleton, rapid water uptake occurs due to a change in osmotic potential. The swelling leads to the highest water contents within the moulting cycle of up to 80% in this phase. In addition to the water uptake, the absence of the exuvia is also responsible for the high water content, as the exuvia accounts for a significant proportion of the dry weight (Perger & Temming 2012). The still soft extremities do not yet allow food intake, which is why the water content only begins to decrease with the onset of hardening of the exoskeleton and a subsequent start of food intake (Hartnoll, 2001). In Stage A, the animals are characterized by a particularly soft exoskeleton, which retains a permanent dent when indented. In Stage B, the exoskeleton remains soft and the water content high, the exoskeleton is still very flexible, but snaps back into its original shape when indented, in this stage the crustacean is often referred to as in “paper firm” condition (Passano, 1960; Perger & Temming, 2012). It is not until Stage C that the water content begins to drop sharply, and the animals can be described as completely hardened. In this stage the exoskeleton cannot be indented easily, so that when using a hard object like tweezers to check the hardness of the carapace, is more likely to cause the carapace to break than to be pressed in.

Due to the very soft exoskeleton in phases A and B, little force was required to pierce the carapace, especially in soft animals, and due to the great flexibility of the carapace, the measurement path until piercing was usually very long, which is why the slope in this phase was equally low and subject to greater variability than slope in harder individuals. With increasing hardening, the carapace offered enough resistance not to be dented before rupture, the measuring distances became shorter, and the force required for piercing increased significantly. *Crangon crangon* shows proportional growth in its body dimensions after completion of the larval phase and hence larger individuals develop a thicker, and thus harder carapace, which requires more force to be ruptured. This can be deduced from the increasing exuvia weight with increasing length (Perger & Temming, 2012; Sharawy *et al.*, 2019). The variations in hardness in supposedly fully cured animals is most likely due to individual differences in carapace thickness which may partly result from different sizes within a 5 mm length class or be related to food intake. The variability in the water content of supposedly hardened animals can be largely explained by the food consumed by the animals. It is known from previous studies that starvation increases the water content of animals (Hufnagl *et al.*, 2010b; Saathoff *et al.*, 2022). The certainly different efficiencies in the intake of the offered food during the curing phase will have led to different water contents after analysis.

Field Samples

Using the length-based thresholds extracted from the laboratory validation experiments of water content, yield strength and slope, the shares of freshly moulted individuals in five field samples of 2021 were identified. Subsequently, this share was used to calculate moulting intervals for each sampling and the length classes 31-35 mm 36-40 mm. Longer moulting intervals were found for larger animals and lower temperatures. This is in line with previous research on the moulting intervall, which was believed to mainly be a function of size and temperature (Hufnagl & Temming, 2011b). Large animals take longer to accumulate the energy necessary for moulting, and lower temperatures slow metabolism, so that the moulting cycle is also slowed. In addition to temperature and size the nutritional status is also crucial for a regular moult (Oh & Hartnoll, 2000). In the absence of food, the energy required for moulting is lacking, and the moulting interval is prolonged until the individual can feed again. The absence of food could explain the longer moulting intervalls observed compared to values found by Sharawy *et al*. (2019), for example in length class 36-40 mm in October 2021 (Tab. 6-5), a month in which primary production decreases due to falling light and temperatures, leading to a limited food supply compared to spring and summer (Reid *et al.*, 1990; Hufnagl *et al.*, 2010b).

For a better comparison of the intervals estimated with the present method with earlier observations the equation provided by Sharawy *et al.* (2019) was applied to our temperature and shrimp length data. The comparison (Tab. 6-5) shows that the interval calculated from frozen field samples, mostly corresponds well to the ones obtained from observations of live shrimp. In some months, slightly shorter intervals are estimated than in Sharawy *et al.* (2019), which could indicate that the animals are capable of shorter intervals than previously assumed. This is supported by more recent laboratory experiments, in which individual observations of significantly shorter intervals than calculated by Sharawy *et al.* (2019) were made.

Table 6-5*: calculated moulting intervals within the catch for the 5 respective sampling month in 2021, compared to latest literature calculations of interval for an theoretical individual of the same size and temperature (Sharawy*

		Length class 31-35 mm		Length class 36-40 mm	
Month	Temperature	Present study	Sharawy *et al.* (2019)	Present study	Sharawy *et al.* (2019)
April	8.1 °C	6.6 days	~ 34.3 days	11.2 days	~ 40.6 days
June	17.3°C	11.6 days	~ 12.1 days	9.5 days	~ 14.4 days
July	18.4°C	8.6 days	~ 10.7 days	12.3 days	~ 12.7 days
August	18.3°C	-	~ 10.8 days	-	~ 12.8 days
October	9.7°C	20 days	~ 28.6 days	34.6 days	~ 33.9 days

The new method showed stronger deviations from the literature values in both length classes of April 2021. These short intervals are unlikely to reflect better feeding conditions. The discrepancy of 6.6 days versus 34.3 days at 8.1°C and 31-35mm length calculated by Sharawy *et al*. (2019) is too high. The relatively high number of freshly moulted individuals in the total catch rather indicates a non-homogeneous distribution of moulting events within the population. This so-called mass moulting, known in other crustaceans, could account for the pattern observed in April. According to reports from fishermen, unusual amounts of freshly moulted shrimp are found right after storm events when water turbidity is high. This observation could also indicate that more animals moult under favorable conditions than usual, which would lead to a non-homogeneous distribution of moulting events in the population.

Methodology

Although the method presented here shows a clear logistical advantage over the conventional determination of the moulting interval in the laboratory, a number circumstances that could complicate the determination must nevertheless be discussed. Firstly does the field sample from August 2021 clearly show that a small sample size of 30 animals is not sufficient to reliably determine an interval. With long expected moult intervals, for example in large animals at low water temperatures, this implies that a large number of animals must be analysed to detect a sufficient amount of freshly moulted animals. However, a further development of the method such that animals moulted within 48 hours can also be reliably identified would enable a calculation of the intervall from a smaller sample size.

Secondly the method runs the risk of overestimating growth, when a non homogenous distribution of moults occurs, or a disproportionate number of freshly moulted animals are accidentally caught during sampling. It is clear that in order to prevent these problems, further research on factors influencing the moulting process of the common brown shrimp is needed. Especially the questions regarding a homogeneous distribution of moults, as well as possible spatial patterns, in which animals use special habitats or water layers for moulting, need further research.

Finally, the determination of the carapace hardness itself should be discussed. During development of the method, different approaches were tried. The force needed for rupturing the carapace was finally used due to its clear signal within the measuring procedure. Unfortunately, no method that kept the carapace intact during measurement and would thus have enabled several readings on the same sample was found to be approximately equal in accuracy as the chosen method. Further trials with blunt indenters, followed by the use of slope only, may allow repeated measurements.

Conclusion

The changing characteristics of body composition and exoskeletal hardness during the moulting cycle of the common brown shrimp has been successfully used to determine recent moults. Following the IGR method, which uses a relative proportion of recent moults compared to the total number of animals, the method described in the present study allows the calculation of the moult interval from frozen field samples by empirically determining the hardness of the carapace. The minor disadvantages of a sufficient sample size outweighs the disadvantages that arise during moult interval determination in the laboratory. In combination with the method of in situ determination of growth increment from field samples (Perger and Temming 2012), the method presented here allows a calculation of a length class specific growth rates without the need of experimental observations on the species.

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Synthesis

A prolonged spawning period, the lack of moult enduring hard structures, as well as the low life expectancy of the species, make age determination of *C. crangon* nearly impossible. Because of this, in conventional age-based management approaches to estimate growth and mortality have been found to be inapplicable, hence to date, common brown shrimp growth experiments have been the primary approach for calculating stock productivity and the potential benefit of conservation measures. Although an abundant selection of growth studies on the species exists, a generally large variability in growth figures within and between those studies as well as some factors influencing the growth of *C. crangon* remain unanswered to date. This thesis addressed four main research objectives concerning the growth of common brown shrimp. Within the thesis a variety of proxies for shrimp growth were used in order to investigate the influence of shrimp density, starvation and cohort effect on growth of the common brown shrimp. Further we developed a method that enables in situ moult interval determination for groups of individuals based on their exoskeleton hardness, which in combination with a method used to determine moult increment, can be used in future growth studies, to evaluate growth, solely based on field samples.

Key Findings

- Mortality induced by starvation in *C. crangon* may be higher, than assumed earlier. Increased mortality was observed primarily after onset of re-feeding, suggesting that the re-feeding after longer periods of starvation seems to be critical in the animal's survival. In contrast to previous studies, the animals showed mortalities of up to 80 % after starvation periods that were significantly shorter compared to existing literature in which the animals were not re-fed.
- The relationship between pre moult dry weight and growth increment (Perger & Temming 2012, Sharawy *et al.* 2019) in common brown shrimp was found to be valid, even when pre moult dry weight is reduced due to starvation. Shrinkage has not been observed in *C. crangon* previously on a systematic basis. Shrinkage due to dry matter loss induced by starvation is most likely a physiological necessity to

ensure homeostasis after moult and could hence be transferable to other starved crustaceans.

- Moulting seems to outrank the compensation of energy lost during food deprivation, since after onset of re-feeding, the animals moulted as soon as the energy necessary for moult was accumulated, regardless of the length decrease after moult.
- Using three different approaches, no indications for a reduced condition or poorer growth performance were found with increasing population density during summer month. On the contrary, we found the best growth performance and highest conditions during summer, in saplings with highest observed densities, suggesting that growth in common brown shrimp is bottom up driven.
- In contrast to density, the seasonal origin of the captured animals could be identified as a key factor influencing growth potential. We found significantly better growth rates in individuals, presumably hatched from winter eggs, compared to ones hatched from summer eggs.
- This observation was found consistently over all observed length classes and during the season. In comparison to Hufnagl & Temming (2011a), we found poorer growth not only in overwintered- but also in summer egg recruits in fall of the same season.
- Growth differences under the same condition and between the two different cohorts were due to both, reduced moult increments and extended intermoult periods.
- The expression of the cohort effect seems to differ greatly between years and to depend primarily on the synchronicity and the characteristics of the individual recruitment waves. A strong intermixing of the different cohorts, for example due to many overwintering summer egg recruits, makes it difficult to assign the animals to a respective cohort and thus reduces the signal of a cohort effect.
- Actual growth observations, and DWCI, an otherwise useful proxy for growth assessment of shrimp, must be used with caution when comparing summer and winter egg cohorts, as mixing the cohorts can easily result in a too blurred picture, and the DWCI depends strongly on the current nutritional situation (higher values in summer than in winter)

- Well growing individuals not only showed large growth increments, but also consistently shorter intermoult periods compared to conspecifics. Individuals that grew poorly on the other hand, showed both small increments and long intermoult periods. The combination of short interval with poor increment, and long interval with large increment, was almost impossible to find.
- Changing properties of the common brown shrimp exoskeleton during the moulting cycle, in combination with dry weight at length (DWCI) were found useful in the determination of recently occurred moults.
- Exoskeleton rigidness is empirically measurable, by measuring the force needed for indention. In recently moulted shrimp, less energy is needed in order to indent and or rupture the exoskeleton, compared to half or fully cured individuals.
- The exoskeleton hardness for a group of individuals could be used in order to estimate, length class specific moulting intervals based on frozen field samples.

Starvation in common brown shrimp- Could density be a trigger?

The following section evaluates the relevance of food limitations in the common brown shrimp stock and for the single individual as well as its potential consequences for future growth calculations. Further the effects of field density as a potential driver for food limitation, on growth and condition of the common brown shrimp will be discussed.

In chapter three we discovered that starvation, followed by a subsequent period of re-feeding significantly reduces growth and increases mortality in common brown shrimp. Although *C. crangon* was thought to cope well with longer periods of starvation (Regnault, 1980; Oh & Hartnoll, 2000; Hufnagl *et al.*, 2010) we discovered, that especially the end of a starvation period, the exact moment when the individual starts feeding again, seems critical in the animal’s survival. We found significantly decreased survival rates after onset of re-feeding which lead to the conclusion, that common brown shrimp will experience higher degrees of mortality due to starvation than previously cited in literature. In survivors, the loss of body own energy reserves during starvation was not compensated for by re-feeding, as previously starving shrimp showed a reduced length of up to 8% after their next moult. We identified shrinkage as a physiological necessity, to cope with lost dry matter during starvation, and to re-establish homeostasis after moulting. We therefore do not rule out the possibility that starvation potentially leads to shrinkage in many more crustacean species than previously known (Nicol *et al.*, 1992; Cockcroft & Goosen, 1995).

Besides being physiologically driven, shrinkage could also be seen as an indirect mechanism to cope with prolonged periods of starvation. While the loss in length is determined by the amount of dry matter consumed during starvation, it could be beneficial to reduce the body size to save energy for metabolism in the event of an ongoing starvation. As with Antarctic krill (Nicol *et al.*, 1992; B. Marinovic & M. Mangel, 1999), the shrinking of *C. crangon* during food shortages can hence also be seen as a type of hibernation technique. Shrinkage has been found occasionally but not been documented systematically in common brown shrimp (Meixner, 1969; Uhlig, 2002; Sharawy *et al.*, 2019), but it may be relevant for accurate growth estimates of the species, especially during winter. Besides the reduction of moult increment due to starvation, we found that the intermoult period which was previously mainly found to be determined by temperature, was significantly altered due to starvation. Since several biological processes in *C. crangon* are linked to regular moulting events, starvation could also have an effect on common brown shrimp fitness, other than by reducing energy reserves and growth rates.

On one hand, it is assumed that the reproduction, and especially the fertilization of the eggs, takes place in freshly moulted females. The spermatophore, which is transferred from the male to the freshly moulted female, is believed to be fertile only a few days after transfer (Boddeke *et al.*, 1991). Further does the egg development time correspond to the moulting interval at a respective temperature (Temming *et al.*, 2017), which shows, that both fertilisation and extrusion of eggs occurs closely after moult. Thus, a prolonged moulting interval due to starvation, in addition to the possible effects on gonadal maturation, could lead to reduced overall productivity in reproductive success. On the other hand, regular moulting prevents infections by for example the black spot shell disease (BSSD) (Segelken-Voigt *et al.*, 2018), more frequently observed in marine crustaceans in the last decades (Stewart, 2021). At the same time, lost limbs or damage to the exoskeleton, such as that caused by contact with fishing gear, can be renewed by moulting.

Field data on dry weight condition suggest that in some months up to 40% of the common brown shrimp stock shows a dry weight condition, which would result in a length decrease if the individuals would moult. Starvation especially during winter was already found by other authors suggesting that average growth rates in winter are close to zero (Hufnagl *et al.*, 2010). Our findings indicate that *C. crangon* may even lose length in this phase. It is noticeable that animals in the laboratory regularly showed negative increments (Fig. 7, points below red dashed line). For animals that were in the laboratory longer before moulting for the first time, the negative increment could be either due to the conditions in the laboratory or the fact that the animals needed some time of ad libitum feeding to accumulate the energy necessary for moulting. Animals of the second category, in combination with those that showed negative increments after a short stay in the laboratory (primarily animals > 40 mm), suggest that negative growth in winter may be more widespread in *C. crangon* than previously known.

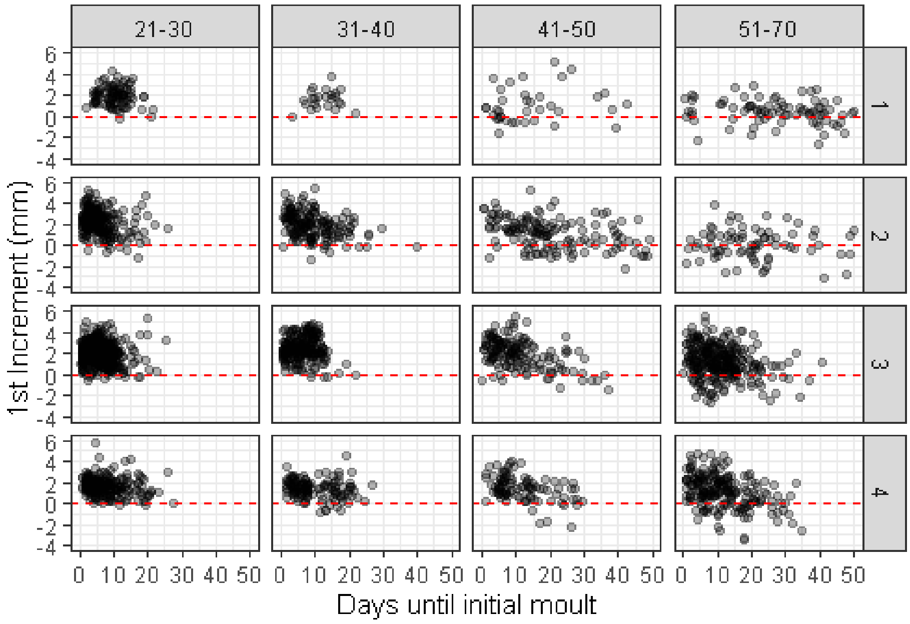

Figure 7: *First increment in mm, over days in the lab until the first moult occurred. A comparison between animals of different sizes and from four quarters of the sampled years between 2018 and 2021 are shown. The red line resembles an equal length before and after* moult. All data points below the red line resemble shrinkage.

Due to intraspecific competition, it can be expected that population density should be a crucial factor in the determination of food availability, and thus, based on chapter three, should directly influence population growth. Especially in situations, in which biomass and hence density will increase, for example due to a sparing management measure, concerns regarding density dependent growth in common brown shrimp are raised. Model calculations have shown that increasing the mesh size in the codend from 20 to 26 mm, for example, would significantly increase both fishermen's catches and the stock size of common brown shrimp (Günther *et al.*, 2021). This could on the other hand reduce growth in a density-dependent manner and negate the effect of a possible increase in catch. In chapter four, we therefore investigated the effects of field density on growth by the means of three different approaches. We specifically investigated the density dependence in summer months, when according to the calculations the greatest possible sparing effect, of a mesh size increase could be expected (Günther *et al.*, 2021).

By comparing either actual growth measurements (Increments), dry weight condition values, or the size compositions of survey catches at different densities, we found no significant decrease in growth potential with increasing density. This was neither the case when investigating different densities within a short time frame, when investigating highly different densities within one season, nor when comparing different densities in individual months but in different years. It appears that good nutritional conditions correlate rather with high densities, and there are no indications that high densities reduce the food availability.

On a local scale migratory behavior of the shrimp could explain the lack of density dependence: in situations when food conditions worsen, shrimp would leave the area leading to a reduction in local density. Vice versa, would a locally restricted increase in productivity lead to an accumulation of shrimp from surrounding areas and to an increase in density. Likewise on a larger scale a productive year would allow many recruits of the common brown shrimp to survive, and thus also lead to high densities. This idea is supported by the fact that all life stages of this very short-lived animal are dependent on primary production (Kuipers & Dapper, 1984). High primary production generates high plankton production and hence optimal survival conditions for larvae and juvenile shrimp (Reid *et al.*, 1990). Further would a strong primary production support the development of detritus - food for benthic organisms- and hence the survival probabilities of also older specimen of the common brown shrimp (Pihl & Rosenberg, 1984; Pihl, 1985; del Norte Campos & Temming, 1994). Hence, high densities should be seen as a positive sign, rather than a potentially growth limiting factor. Local abundance as well as population size could therefore be considered bottom up regulated. Furthermore, it was shown that the natural variations in density, both when using data of only one sampling site and based on commercials or DYFS Survey data, far exceeded the expected increase in density resulting from the proposed management measures such as a mesh size increase from 20 to 26 mm of around 23% (Günther *et al.*, 2021). Therefore this counter argument is no longer valid. Nevertheless, the combination of results from chapters 3 and 4 indicates a potentially critical nutritional situation in the adult population in winter. Regarding the natural density differences in the stock, it would be prudent to refrain from reducing the stock in winter by increasing effort to avoid potential density dependence. Rather, it appears that food is scarce regardless of stock size, and increased fishing pressure would result in fewer animals in equally poor condition. The additional effort would mainly target the fitter animals that still perform a tail flip and in addition increase discard mortality.

Cohort effect

The following section describes growth differences between same sized individuals of the common brown shrimp, held under the identical conditions but caught at different times within one season. The subsequent analyses was based on a phenomenon known as the cohort effect, which was first detected in a 2011 growth study on *C. crangon* and was subsequently not further investigated. In 2011, Hufnagl and Temming studied animals of the same size caught only about six weeks apart (animals between 20-30 mm, from the months of May and July). They found growth rates close to zero in the May animals, and in those caught in July at the same size, ones exceeding all previously documented growth rates. The authors attributed this, after excluding all external factors, to the seasonal origin, i.e., the age and feeding history of the captured individuals. Individuals caught at 20-35 mm in May were most likely summer egg recruits of the previous year. Compared to the individuals caught in July, may individuals would hence have experienced long periods of poor food availability during winter (Hufnagl & Temming, 2011a).

In chapter five we investigated the effect of sampling month, and hence seasonal origin of the caught individuals, on their growth rates. Like Hufnagl and Temming, we found significantly better growth performance in winter egg recruits, compared to recruits hatched in summer. We did not only find poorer growth performance in overwintered-, but also in summer egg recruits caught in the same season they hatched. The significantly poorer growth performance was recognizable, both via shorter moult increments and extended intermoult periods. We further found that growth differences related to the seasonal origin could be confirmed for almost all length classes investigated.

In Hufnagl and Temming (2011) the hibernation and potential irreversible non-genetic effects in summer egg recruits was primarily mentioned as the possible reason for the large differences in growth potential between the two cohorts. Our observed growth differences can probably also be explained by irreversible non-genetic effects induced by the nutritional history of the respective cohort (Kinne, 1962). Winter egg recruits are known to hatch from significantly larger and more energy rich eggs (Boddeke, 1981; Hünerlage *et al.*, 2019). Winter egg larvae are therefore predicted to have a higher fitness compared to summer egg larvae (Paschke *et al.*, 2004; Urzúa & Anger, 2013). At the same time, their hatching in March is synchronized with the first plankton bloom occurring in the southern North Sea which provides perfect growth and survival conditions for the animals (Reid *et al.*, 1990; Temming & Damm, 2002; Urzúa & Anger, 2013). Summer egg recruits, on the other hand, hatch from smaller, less energy rich eggs. They hatch in a system with a rapid turnover, high predation rates and high energy requirements at high water temperatures. A possible adaptation of the respective cohorts to their early-life growth performance, could lead to the differences between the two later in life. For an individual that has reached a length of 30 mm late in the season, e.g. in September, it would be advantageous to enter the winter period with poor food availability at a shorter length. Sexual maturity and the ability to reproduce (the ultimate goal) will not be reached before winter. Therefore, slower growth of the summer eggs recruits, which they experienced from the start of their life, could be an advantage. An advantage that seems to outweigh even the other benefits that fast growth would bring, such as escaping the prey spectrum of various predatory species.

The experimental period from late 2018 to 2021 showed that the cohort effect can vary in strength between different years. Possible reasons for this could be the strength of the respective recruitment waves between the different years. If recruitment of a respective cohort turns out to weaker than usual, it´s appearance in both commercial catches, and growth observations will be masked by the higher number of individuals of other cohorts existing in parallel. We suspect that the growth anomaly from 2019, which was reflected in both growth trials and commercial fishery catches (ICES, 2022), was due to such a phenomenon. Quarter 1 of 2019 was already characterized by high densities of individuals in a 20-40 mm size range, suggesting that many summer egg recruits from 2018 survived the winter. The large number of hibernated individuals might have reduced the otherwise high survival chance of the winter egg recruits in spring 2019, resulting in a more or less complete failure of the winter egg recruitment. As the season progressed, overwintered summer egg recruits from 2018 mixed with new summer egg recruits from 2019 which in turn would explain the generally poor growth performance of all length classes throughout the 2019 season.

Variability in growth – Interaction of increment and interval

During the growth trials between 2018 and 2021 a novel achievement was the measurement of individual growth rates, with both moulting interval and moult increment observed for single individuals. We found that the period between two successive moults and the size of the increment after moult are somewhat closely related. During each of the experimental runs, we experienced that well growing individuals not only showed large growth increments, but also consistently shorter intermoult periods compared to conspecifics. Individuals that grew poorly on the other hand, showed both small increments and long intermoult periods. The combination of short interval with poor increment, and long interval with large increment, was almost impossible to find. In all observed length classes, the animals could be distinguished into winners, animals that showed large increments within a very short time, intermediate growers with both medium increments and intervals, and losers that showed longer intermoult periods at small or no increase in length (Fig. 8).

When comparing animals of the same length kept under the same conditions (same temperature, same setup, and ad libitum feeding with the same type of food), three of the five factors that influence growth detected by Hufnagl and Temming (2011) should be excluded in distinguishing winners from losers. While size and temperature certainly can be, food availability cannot. Although all individuals observed where fed the same ad libitum diet, the actual food intake in *C. crangon* is difficult to quantify. *C. crangon* shows its primary activity at night and feeds mostly here. At the same time, it dissects its food while feeding, and with larger food particles it is difficult to determine how much of it the shrimp ultimately ingested.

We found that individuals in the laboratory responded quite differently to the food offered to them. While some of them accepted larger quantities in short intervals, especially larger animals often showed no interest in the offered food (Haghani, 2021; Meier, 2022). It is therefore possible that finding a way to quantify the actual food intake, will help excluding losers that just haven't fed well during their stay in the laboratory. Regarding cohort, we could prove, that months in which the likelihood of investigating primarily winter egg recruits was high (e.g. length class 20-30 in July), the share of winners was large compared to months in which mostly, presumable summer egg recruits were investigated (e.g. length class 20-30 mm in September). From the experiments with larger animals, in which also the sex of the animals should have had a greater influence on the growth (Hufnagl & Temming, 2011b), we found primarily females being winners, whereas the males were rather intermediate growers or losers.

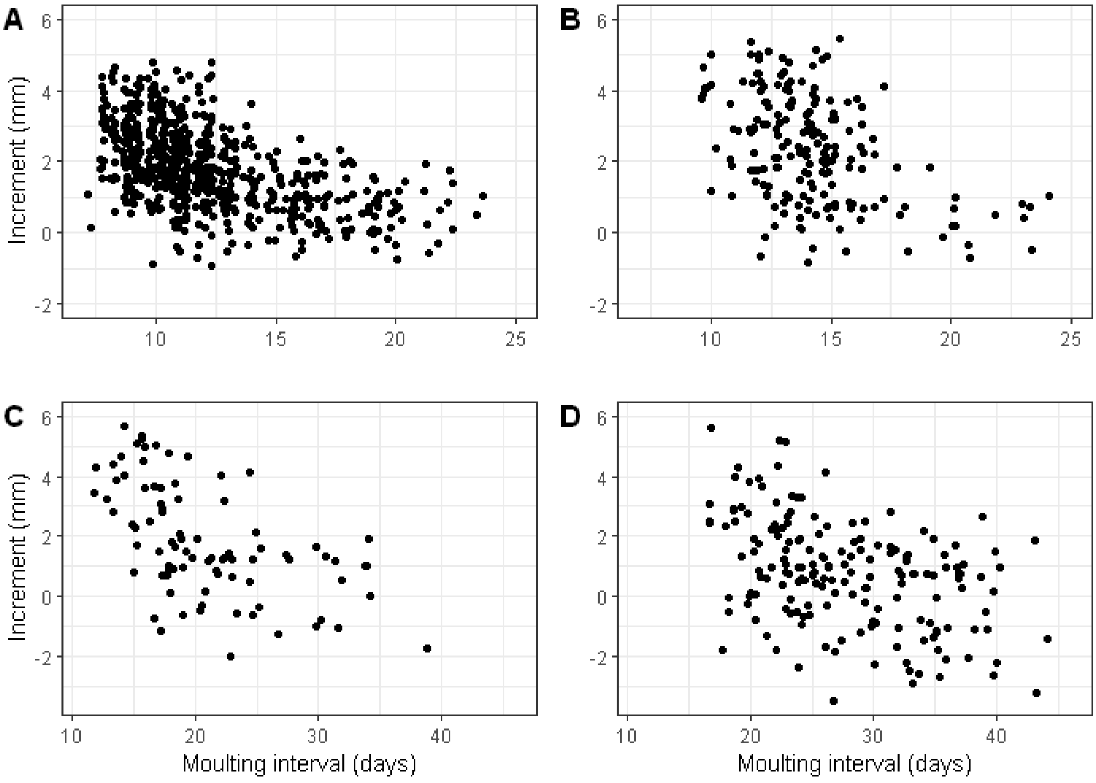

Figure 8*: Moult Increment in mm over moulting interval in days for 4 Length classes. A= 21-30 mm, B= 31-40 mm, C= 41-50 mm, D= 51-70 mm (17°C trial temperature)*

Growth of Individuals larger 50 mm

Compared to smaller individuals investigated, animals larger than 50 mm grew poorly in all conducted experiments between 2018 and 2021. Figure 9 illustrates that especially the increments decreased significantly with increasing animal size. Already in the growth studies of other authors it was noticed that especially adults are difficult to maintain in the laboratory, and large growth rates of more than 0.3 mm per day could actually only be observed in animals smaller than 40 mm (Hufnagl & Temming, 2011b; Delbare *et al.*, 2015; Meier, 2022). We suspect that this is due to the fact that in the laboratory no adequate food can be made available for the larger animals. While small individuals respond well to live nauplii of *Artemia Salina*, and live copepods (Meixner, 1969; Hufnagl & Temming, 2011a), these particles seem too small for animals larger than 40 mm (Pihl & Rosenberg, 1984; Pihl, 1985; del Norte Campos & Temming, 1994). It appears that especially living food animates the shrimp to feed, which leads to the animals being inactive when frozen but otherwise rich food, or commercial fish pellets are offered (Haghani, 2021; Meier, 2022).

While it was difficult to animate larger individuals to show higher growth increments reported by other authors, figure 9 b shows that moulting intervals of these individuals can be much shorter than previously observed. The red dashed line indicates a predicted moulting interval at maximum growth, based on the most recent moult interval observations on common brown shrimp (Sharawy *et al.*, 2019). While the prediction matches our observed moulting intervals of individuals shorter 35 mm, many of our larger animals show much shorter intervals than predicted. In Sharawy *et al*. (2019) the moulting intervals were determined using the IGR method, i.e. a fraction shrimp that moulted after a few hours in the laboratory (48 h) was used to calculate an interval for an entire length class. Our results in comparison are based on actually measured intervals. The fact that the animals are capable of significantly shorter intervals than previously assumed suggests, on the one hand, that with high increments, larger animals would also be capable of significantly higher growth rates than previously assumed, and on the other hand, that the moulting interval should be given significantly more attention when determining growth potential.

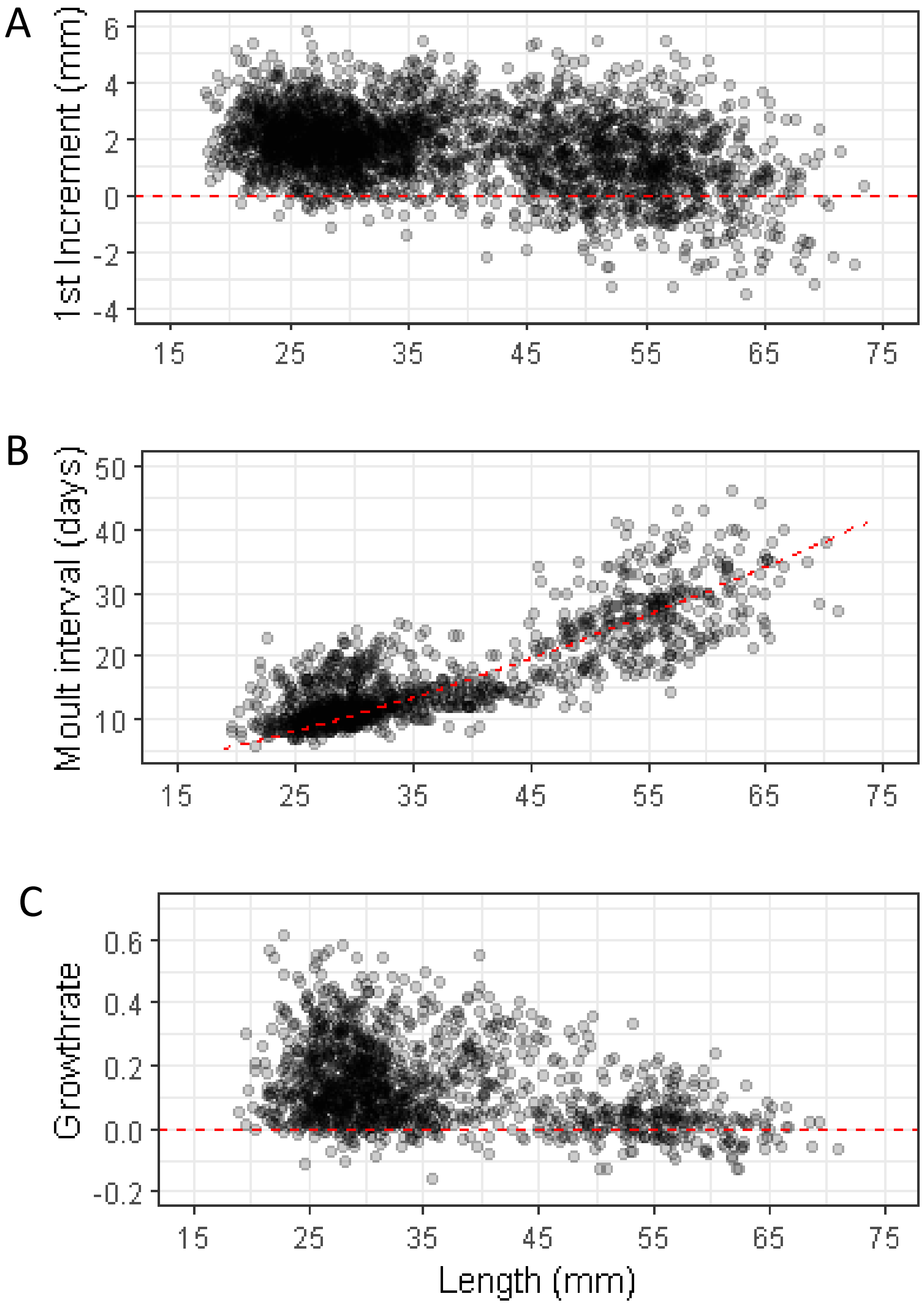

Figure 9*: First increment (A), Moulting interval (B) and Growth rate (C) over Size. Red dashed line in A and C = zero growth, in B= Moulting interval calculated based on Sharawy et al. (2019) for individuals held at 17 °C*

New method of determining moulting interval

To make moulting interval observations more accessible in the future, while addressing any inconsistencies associated with animal being kept in the laboratory, we have devoted chapter 6 to the development of a method for the in situ determination of the moulting interval from frozen field samples. Already with the instantaneous growth rate (IGR) method, long laboratory experiments to determine actual moulting intervals could be avoided. Instead, moulting interval for a group of individuals (e.g. a length class) were based on the proportion of animals moulted within a short period (1 – 2 days) in relation to the total number of animals within the group (Quetin, L. B., Ross, R. M., & Clarke, 1994). In contrast to the IGR, we wanted to identify the proportion of freshly moulted animals within a group, directly from a frozen field sample, and without the expense of a laboratory trail.

We developed a device to detect shrimp that moulted within the last 24 hours from frozen field samples. This required a series of calibration experiments in which the moult event of common brown shrimp was registered to the minute. This was necessary because the properties of the exoskeleton change rapidly after the moult. In the crustacean moulting cycle, dry weight oscillates constantly between periods of high dry weight prior to moulting, and periods of lowest dry weight immediately after moulting (Hartnoll, 2001; Perger & Temming, 2012). We used this in combination with the hardness of the exoskeleton, which is very soft especially immediately after a moult, to detect freshly moulted animals in frozen field samples. We found that the moult interval for a group of individuals could be determined consistently, as the calculated intervals could be well matched with moult intervals predicted under the same environmental conditions by other authors. The ability of the newly developed method to determine moulting interval on the basis of a single field sample, without prolonged husbandry trials, will further improve the understanding of growth of the common brown shrimp. With sufficient sample size, the new method, in combination with a method to determine moult increment based on frozen field samples (Perger & Temming, 2012; Sharawy *et al.*, 2019), will allow for an easy length-specific growth rate determination, and thus contribute to the understanding of previously less well studied phenomena such as the cohort effect, or adult shrimp growth.

Conclusions and management perspectives

- Starvation in the common brown shrimp stock is common, and at all times during season, individuals can be found that show dry weight conditions usually found only in starved individuals. We can conclude that in each experimental run, the likelihood having included at least a few previously starving individuals is high. Since ad libitum feeding does not seem to increase growth after a starvation period, all subsequent growth observations may be biased by the unknown share of starved individuals.
- The reduced growth during the winter season due to starvation should be considered in the growth sub-model of the Y/R simulation model (Temming *et al.*, 2017; Günther *et al.*, 2021).
- Estimated growth rates of larger individuals may be overestimated in previous investigations
- No evidence was found that higher densities reduce growth rates in the field even with very large differences in field density. In addition the natural variation in density is much larger that the small density increase that is expected after the increase of mesh sizes to 26 mm.
- Winter egg recruits grow better than summer egg recruits. Assuming that adults originating from winter egg recruits contribute most to the common brown shrimp fishery in fall, protection specifically of the winter egg cohort should be recommended in future management approaches.
- Exoskeleton properties, in combination with body composition properties, can be used to estimate moult intervals frozen individuals of c. crangon.

Future research perspectives

- The generally poor growth performance of larger common brown shrimp in the laboratory, in combination with the observation of shorter moulting intervals than previously observed, indicates the need for further growth trials, especially on larger individuals of the common brown shrimp. In order to exclude laboratory effects, the focus should be on individuals that moult immediately after capture (Instantaneous Growth Rate Method).

- The newly developed method for the in situ determination of the moult interval should be applied in combination with the method for the in situ determination of the growth increment (Perger & Temming, 2012) to map the growth in *C. crangon* of different sizes within the season and based on field samples. This will also contribute to the better understanding of the cohort effect.
- The long-term consequences of starvation periods in C. crangon require more attention given the high frequency of shrimp found in poor condition. If non-genetic irreversible adaptation occurs in common brown shrimp is yet to be proven.

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Outline of publications

The following overview outlines the four publications included in this thesis and the contribution of the co-authors to the manuscripts. The overall objectives of this research was embedded in the project: „Wissenschaftliche Untersuchungen zur Biologie und Fischerei der Nordseegarnele CRANgon crangon als Basis für ein effizientes Selbst-MANagement Systems (CRANMAN) “.

Manuscript 1: Effects of starvation and subsequent re-feeding on moulting interval, growth increment and mortality in common brown shrimp (*Crangon crangon*).

Merten Saathoff, Axel Temming, Robert Perger

Merten Saathoff performed the growth experiments from 2017 and the literature research and wrote the manuscript. Pre-Experiments from 2008 were performed by Robert Perger. Rober Perger and Axel Temming helped with the writing and the design of the experiments. The manuscript was submitted to Marine Biology a peer review Journal.

Manuscript 2: Density dependence in common brown shrimp (*Crangon crangon*), a three-way approach

Merten Saathoff, Holger Haslob, Magarethe Nowicki, Axel Temming

Merten Saathoff performed the growth experiments, the literature research and wrote the manuscript. DYFS Data were pre-aggregated and supplied by Holger Haslob. The Analysis on DYFS and commercial fisheries data was performed by Magarethe Nowicki. Axel Temming helped with the writing and the design of the experiments.

Manuscript 3: Cohort Effect in common brown shrimp (*Crangon crangon*) – Differences in growth potential between shrimps of different seasons

Merten Saathoff, Antonia Uthoff, Axel Temming

Merten Saathoff performed all growth experiments, except ones from April May and June 2019, which were performed by Antonia Uthoff. Merten Saathoff conducted the literature research and wrote the manuscript. Axel Temming helped with the writing and the design of the experiments.

Manuscript 4: Establishing a method for in situ determination of the moult interval and moult stage from frozen field samples of shrimp. A case study on the common brown shrimp (*Crangon crangon)*.

Merten Saathoff, André Harmer, Sven Urban, Axel Temming

Merten Saathoff performed the validation experiments, the validation- and actual measurements, the literature research and wrote the manuscript. Sven Urban and André Harmer helped in in the development, construction and programming of both the validation setup and the hardness measuring device. Axel Temming helped with the writing and the design of the experiments. The manuscript is ready to be submitted to the peer reviewed Journal of Experimental Marine Biology and Ecology.

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Eidesstattliche Versicherung - Declaration on oath

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, den 19.01.2023