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D1.4 Meta analysis of the driver databases and development of new parameterisations relevant to the ecosystem models

Part F: Multi driver responses

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This section is composed of two parts. Firstly a literature review of the impacts of multiple stressors on marine plankton and secondly a statistical meta-analysis of the experimental results (T1.3) of organismal and ecosystem response to the drivers. For details of the experimental activities leading to these results you are referred to D1.6

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1. The effects of natural multiple stressors on vital rates of marine plankton organisms

1.1 Introduction

A number of environmental changes as well natural climate induced changes, are predicted to affect world's oceans. These changes are mainly examined for abiotic factors such as temperature, light, turbidity, acidification or salinity, but also for biotic stressors, primarily predation and food availability. These factors – also known as climate drivers – may highly fluctuate in marine ecosystems. They do not merely influence each other but rather the whole oceanic living environment.

We do know a lot of single stressors influencing different marine organisms, but in contrast little is known about how organisms might react on multiple stressors. This lack of knowledge may be due to the fact that combined effects of two or more variables are more difficult to predict than individual effects, as the impact of one factor can intensify the impact of another. Already FOLT ET AL. (1999) showed such an antagonistic effect of multiple stressors on marine plankton organisms' vital rates as most frequent, while weakening effects have only rarely been observed.

The influence of various stressors can push a population or an individual beyond a critical threshold that would not be reached in this extent by one stressor alone (HARLEY ET AL. 2006). In fact, most aquatic systems are threatened by multiple stressors (HUGHES AND CONNELL 1999, VINEBROOKE ET AL. 2004). Therefore, more knowledge about the interplay of several stressors is required.

The purpose of this review is to analyze interactions between different combined biotic and abiotic stressors acting on marine zooplankton organisms. Based on articles in the period of 1970 to 2010, we reviewed twenty-six studies referring to two or more combined natural stressors affecting marine zooplankton.

The most investigated taxa among all reviewed articles, are mainly copepods and different developmental stages of other crustaceans. Even though crustaceans are one of the most important organisms in the marine food web, providing food for many planktivorous organisms, e.g. fish larvae, is marine plankton more diverse. Therefore, we will include a few meroplanktonic organisms such as sea urchins, polychaetes and fish with planktonic larvae. Since a large part of the literature examines different factors combined with salinity or temperature, other abiotic factors, such as turbidity, oxygen and CO₂ level or biotic factors as food availability and predation are underrepresented, but not less interesting.

The most investigated and important climate driver is temperature. Previous studies showed direct effects of increasing water temperature on shifts in abundance, distribution and growth of some fish species and their planktonic prey (e.g. VAN HAAL ET AL 2009, WILTSHIRE ET AL 2004). Many chemical and biological processes, such as metabolic rates, growth, reproduction, survival and uptake of toxic substances, are indirect effects influenced by temperature changes. (STRICKLAND 1965; HOUSTON 1982; AIKEN AND WADDY 1990). Especially poikilotherm organisms (which cannot regulate their internal temperatures), such as marine plankton, are strongly affected by these changes.

Even though Temperature seems to be most important, other factors as salinity, turbidity, predation or food availability can cause limits in vital rates and in this way also in stocks and whole populations. For the performance, survival and dynamics of many plankton organisms, changes in chemistry as well as ocean circulation might be more important (HARLEY ET AL. 2006), causing plankton distribution and transport of nutrients and food. Just as stressors, e.g. temperature and salinity operates on chemical processes such as uptake of pollutants (BRYANT ET AL. 1985; LEVENT ET AL. 2000) or physiological processes like excretion and respiration (GAUDY ET AL. 2000, are multiple stressors operating on a number of physiological processes.

1.2 Response to Salinity and Temperature

Salinity is known to be an important factor in oceans chemistry. Stratification and distribution is strongly influenced by salinity and temperature. Thus changes in salinity will have an impact on marine environments biodiversity.

An example on influences of salinity-temperature interaction is given by HOLSTE AND PECK (2006) In this study, one of the most frequent copepods in the Baltic Sea, *Acartia tonsa* showed an increasing hatching success of incubated eggs with increasing salinities between 17 and 25 psu. A good example on high impacts of changes in salinity is referred by MÖLLMANN ET AL. (2000). They assume that decreasing salinity might cause a regime shift in the dominant copepod species in the Baltic Sea. The former species *Pseudocalanus elongatus* and *Pseudocalanus acuspes* require a higher salinity for a more successful reproduction than species of *Acartia* (mostly *Acartia longiremis* and *Acartia bifilosa*).

Temperature increase and changes in salinity are known to strongly affect metabolic rates and thus postembryotic development (GILLOOLY ET AL., 2002; NAGARAJ, 1988). Previous environmental conditions hardly influence the tolerance of these organisms. For *T. longicornis*, this has been revealed in different studies in North Sea and Baltic Sea populations (e.g. PETERS 2006, HALSBAND ET AL. 2001, 2002). HOLSTE ET AL. (2009) found significant differences in egg production and

hatching success when *T. longicornis* was exposed to varieties of salinity after acclimation to different salinities under laboratory conditions. Egg production was always highest at the acclimation salinity.

For *Temora brevicornis*, a high-shore harpacticoid copepod, DAMGAARD AND DAVENPORT (1994) examined an enhanced tolerance to high salinity when acclimated to higher salinities and low salinity tolerance was nearly unaffected by acclimation salinity. Salinity preference experiments demonstrated the ability of copepods to choose salinities near their range of acclimation. Copepods acclimated to rather extremely high or low values favored more natural conditions. They are able to differ in a range of 3 ‰.

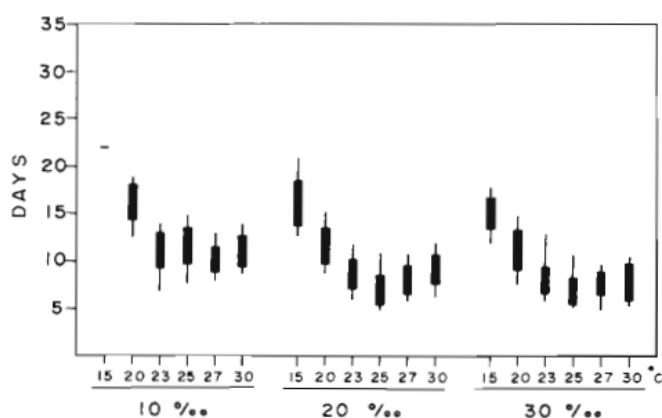


Figure 1.2.1 shows the time (d) required by the nauplii of *Balanus amphitrite* to reach the cyprid stages at different salinity-temperature conditions. Bars indicate mean SD with lines indicating the range. (ANIL ET AL. 1995).

However, if we consider vital rates being influenced by different combinations of salinity and temperature, a general prediction is more complex and almost impossible. Apparently, lower temperatures causes a higher salinity tolerance in some organisms. In the case of copepods, DAMGAARD AND DAVENPORT (1994) examined that the survivable salinity range for *T. brevicornis* (Harpacticoid) was higher for individuals acclimated to lower temperatures and an acclimation to higher salinities increases survival of higher temperature. This was earlier described by NAGARAJ (1988) in the case of *Eurytemora velox* (Calanoida). HOLSTE ET AL. (2009) showed that naupliar mortality of *Temora longicornis* increased with increasing temperature from a range of 26.7% at 10° C to 63.2% at 20° C within a salinity of 7‰. At a psu value of 20, mortality ranged from 1.7% at 10° C and ≤ 26.7% for all other tested temperatures (10, 12, 14, 16, 18 and 20° C).

An example for the complex interrelation of these two parameters is revealed by ANIL ET AL. (1995) in an experiment with larvae of the barnacle *Balanus Amphitrite* (Cirripedia) collected in a semi-enclosed environment with limited water exchange in the Pacific Ocean (Hamana Bay, Japan). Larvae were reared at temperatures of

15, 20, 23, 25, 27 and 30°C and salinities of 10, 20 and 30‰. This study represents another example of adaption to changing environmental conditions.

As described by e.g. CRISP & COSTLOW (1963), is *B. Amphitrite* an eurythermal organism, which can breed at temperatures of 15° C. In the study of ANIL ET AL (1995) development of *B. Amphitrite* larvae into the cyprid stage were successful at all tested salinities.

The best salt-temperature conditions for larval development seem to fall in a range of 20 to 25° C, where development duration was shorter (Fig. 1.2.1). At 20‰ and 30‰ the average development duration ranged from 7.1 to 16.3 days and was longest for 10‰ with an average of 11.1 days at 30° C and 22 days at 15° C. Mortality rates were considerably more affected by temperature than by salinity. In general, temperatures of 15 and 20° C caused a higher mortality in this barnacle species than higher temperature. The lowest mortality rate occurred at a temperature of 23° C for all salinities and at 27° C for a salinity of 30‰. This shows that *B. Amphitrite* is able to breed at lower temperatures, but development is slower and mortality is higher in dependence of salinity-temperature interaction.

In Deverker et al. (2007) the situation is just as complex as the conditions above. The authors investigated Post-embryonic development time of another common calanoid copepod, *Eurytemora affinis*, caught in oligo- and meso-haline zones (salinity 2.5–25) in the Seine Estuary. Development time of nauplii, copepodites, egg to adult and lifetime length in six conditions (temperatures 10 and 15°C combined with salinities of 5, 15, 25 ‰) were tested (Fig. 1.2.2). Mean postembryonic development time decreased from 24 to 17 days at increased temperatures from 10 to 15° C. As in HOLSTE ET AL. (2009), an increase in mortality and development time was caused by an increase of salinity. A salinity of 25‰ was suggested to be the most stressful of the tested conditions, while no significant difference in development time or mortality was observed between salinities of 5 and 15 ‰. With regard to individuals of *E. affinis*, the influence of multiple stressors it is getting more complicated. Individual developmental variability was very high and increased during development. Hence, some individuals were slower at salinities of 5 and 15‰ than at a salinity of 25‰. (Deverker et al. 2007).

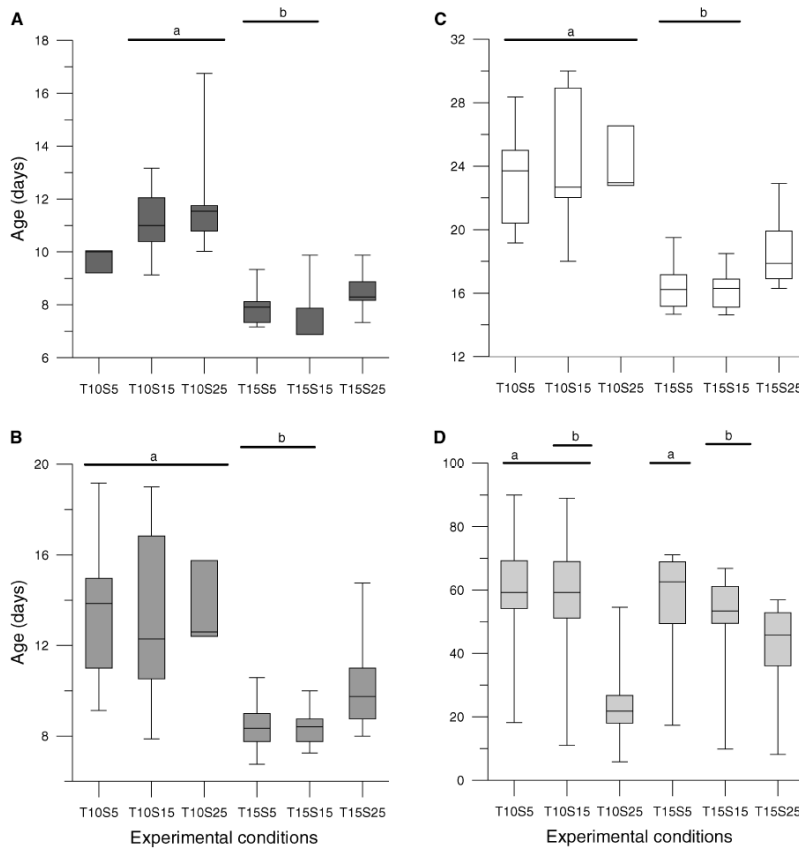


Figure 1.2.2: shows Whisker-Box plots with median and percentiles. A = development time of nauplii, = copepodites, C = egg to adult, D = lifetime length in the six experimental conditions. a or b groups are not significantly different (tested with Wilcoxon analysis with significant level at P , 0.01). (Deverker et al. 2007).

Not only crustaceans are influenced by a salinity-temperature interaction. Another example, the planktonic larvae of *Marenzelleria viridis* (Polychaeta, Spionidae), was investigated by BOCHERT ET AL. (1996). To observe larval development, naturally spawned eggs were collected at a temperature of 14.8°C and a salinity of 5.9‰, south of the Darss-Zingst in the Baltic Sea (54°25'N, 12°40'E).

They were raised under laboratory conditions at different temperatures ($5 \pm 0.3^\circ\text{C}$, $9.3 \pm 0.05^\circ\text{C}$ and $19.9 \pm 0.3^\circ\text{C}$) and salinities ($0.6 \pm 0.04\text{‰}$, $2.6 \pm 0.1\text{‰}$, $5.1 \pm 0.2\text{‰}$, $10.3 \pm 0.3\text{‰}$ and $20.1 \pm 0.5\text{‰}$). Under natural conditions, *M. viridis* spawn at water temperatures below 15°C. During the larval phase, water temperature decreases under 0°C (BOCHERT AND BICK, 1995). Development of *M. viridis* larvae in the laboratory was shorter and showed higher growth in length at a temperature of 20 °C than at 10 °C, but at 20 °C larval development showed abnormalities in growth of larval spines and different types of cilia. In consequence they are not viable. (BOCHERT ET AL. 1996).

Most zooplankton organisms are able to survive under changing climate conditions, but high vital rates are important for long-term maintenance. According to their prevailing natural requirements,

the investigated organisms have different temperature and salinity optima for successful reproduction and development. In the case of the two most common copepod species in the Atlantic and Mediterranean, *Acartia tonsa* and *Acartia clausi*, an influence of habitual (acclimation) temperature and salinity conditions are verified. GAUDY ET AL. (2000) measured respiration rates and ammonia excretion in different temperature-salinity combinations. The respiration rate tells us how much energy an organism can get out of their food. Some of the energy is used directly for basic functions like ingestion or growth. Since adult copepods do not moult, they are very efficient in transforming ingested material into eggs. According to the energy balance equation, reproduction is directly related to food acquisition and inversely to metabolic expenditures. (KIØRBOE ET AL. 1985).

The Influence of salinity-temperature conditions on respiration and excretion in copepods is described by GAUDY ET AL. (2000). *A. clausi* and *A. tonsa* are good examples of organisms with differences temperature-salinity optima, referred to their habitual conditions. For *A. clausi* GAUDY ET AL. (2000) occurred lower respiration rates at a salinity of 35‰ in different temperatures (10, 15 and 20° C) as for in *A. tonsa*. The opposite has been observed at low salinity (15 ‰).

This can lead to different acclimation potential as a consequence of the animals' origin. *A. tonsa* was originated of a semi-closed brackish area (Etang de Berre) in the French Mediterranean Sea with an average salinity of 15‰. In contrast, *A. clausi* was acclimated to a salinity average of 35‰ in the Gulf of Fos. On the other hand, other copepod species or populations might have the ability to adapt more spontaneously and cope better with a changing environment. One example is the copepod *Eurytemora affinis*, an invasive, predominantly estuarine and salt marsh organism, which can tolerate salinities up to freshwater level (LEE ET AL. 2003). The population of *E. affinis* from the Seine estuary showed a faster development rate than conspecific populations from other estuaries at similar experimental temperatures and salinities (DEVERKER ET AL. 2007).

As mentioned above, reproduction in copepods directly related to food uptake and indirectly affected by metabolic expenditure, which in turn is influenced by temperature and salinity conditions (GAUDY ET AL. 2000). A more detailed view on the influence of food availability, predation and other dietary relevant factors are discussed in the following.

1.3 Response to Temperature, Salinity and Food availability

Temperature is one of the main parameters which have an influence of zooplankton vital rates if food availability is optimal. But since ingestion rate in copepods varies with food concentration (e.g. FROST 1972) and discontinuous food availability has an effect on egg production (DAGG 1977), a combination of these factors might have a stronger effect than expected.

HALSBAND- LENK ET AL. (2004) compared two copepod species, *Temora longicornis* and *Centropages hamatus* in two different marine environments (North Sea and Mediterranean) during a multi annual period. They revealed that reproduction in the North Sea (Helgoland Island) was independent of the food conditions. These findings are contrasting to the set-up in the Bay of Villefranche (Mediterranean), a more temperate and oligotrophic area. Thus, food limitation led to food competition and seasonal separation. This had a negative effect on reproduction rates and body size. Former results are based on natural conditions in the Mediterranean and North Sea. Under natural conditions, other factors than the examined ones might play a role. To identify a more exact correlation between nutrition as a part of a multiple stressor system, it is necessary to study different organisms under standardized laboratory conditions.

To start with a low trophic organism, we are looking at survival and fecundity of the marine rotifer, *Synchaeta cecilia valentine*. This rotifer is a major food source of fish larvae and other zooplankton organisms as well in nature as in aquacultures. OLTRA AND TODOLF (1997) isolated a strain of *S. c. valentine* from the Mediterranean (salinity of 22 ‰) and cultured them in the laboratory at two temperatures (20 and 24°C), four salinities (20, 25, 30 and 37‰) and two food levels of a suspension of the algae *Tetraselmis suecica* (15 000 and 25 000 cells ml⁻¹). As discussed above, might high temperature and high salinity have a negative effect on survival and development (e.g. Deverker ET AL. 2007; HOLSTE ET AL. 2009). Evidently it is the same in the following study of (OLTRA AND TODOLF 1997). Higher Temperatures or higher salinities have a significant negative effect on the average lifespan and on the number of offspring of *S. c. valentine* ($P < 0.001$). The lifespan was in a range of 10-11 days at a temperature of 20°C and a salinity of 20 and 25‰, varying between 8 and 10 days at 24°C and a salinity of 20 ‰) (compare to fig. 1.3.1).

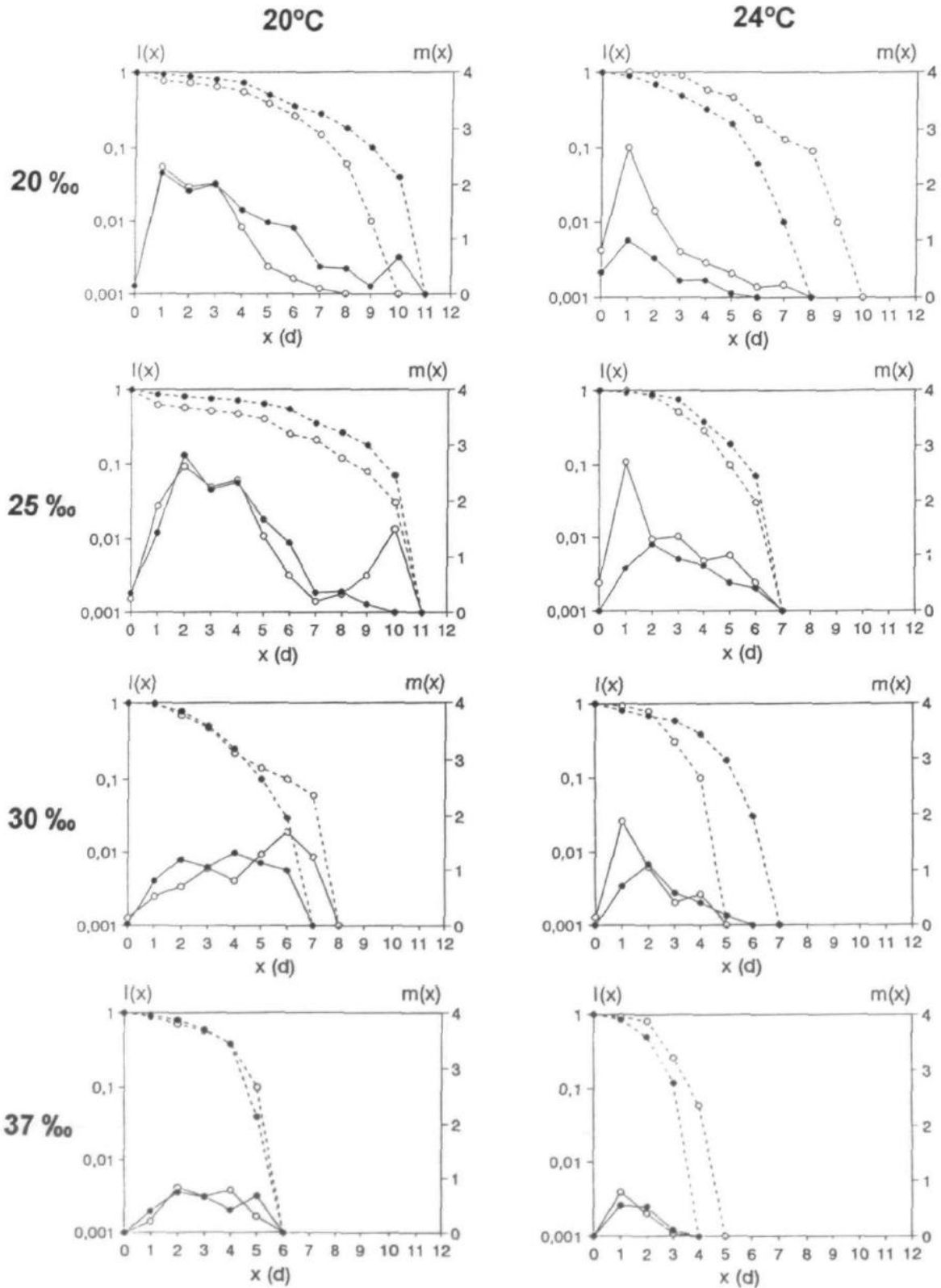


Fig. 1.3.1 shows Age-specific survival $l(x)$ (dashed line), and fecundity $m(x)$ for *Seccilia valentina* grown at different temperatures (20 and 24°C), salinities (20,25, 30 and 37‰) and food level closed circles (15 000 cells ml^{-1}) open circles (25 000 cells l^{-1}), Age (x) in days (d). (OLTRA AND TODOLF 1997).

A negative effect on lifespan and growth rate is also known from other rotifers, e. g. *Synchaeta littoralis* (BOSQUE ET AL 2001) and *Brachionus plicatilis* (SERRA ET AL. 1994). A positive effect of certain temperature-salinity combinations has been found for combinations with low or high values for both factors. R_0 (average offspring per female) ranged from 8.5 to 9.1 offspring per female at 25‰ and 20° C and from 4.2 to 4.4 at 35‰ and 25°C. This range is typical for rotifers natural environment (MIRACLE AND SERRA 1989). Adequate effects are revealed for fish and some invertebrate species by ALDERDICE 1972 and KINNE 1970 cited in MIRACLE AND SERRA 1989.

In a combination of all three stressors, temperature, salinity and food, the impact on growth is obviously dependent on food concentration. This was for example revealed for *S. c. valentine* by OLTRA AND TODOLF (1997). At 20°C, the number of offspring per female and their growth rate has reached its maximum at a lower algae concentration (15 000 cells ml⁻¹) while a higher food concentration was needed to reach the maximum at a temperature of 24° C. As much as effects on growth rate and reproduction might be distinct, just as unclear is the effect of food in context with salinity and temperature on lifespan. For *S. c. valentine*, the maximum lifespan was recorded at 20°C, salinity of 25‰ and at the lowest tested food concentration. In contrast, the lifespan of *Synchaeta littoralis* (BOSQUE ET AL. 2001) increased with food level and their maximum lifespan was seemingly shorter in higher salinity and temperature conditions (Fig. 1.3.2). Only in the trial with a salinity of 30‰ and a temperature of 20° C, the average lifespan decreased from 5.4 to 5.2 days with rising food concentration (from 75000 to 150000 cells ml⁻¹), which does not fit in the overall pattern.

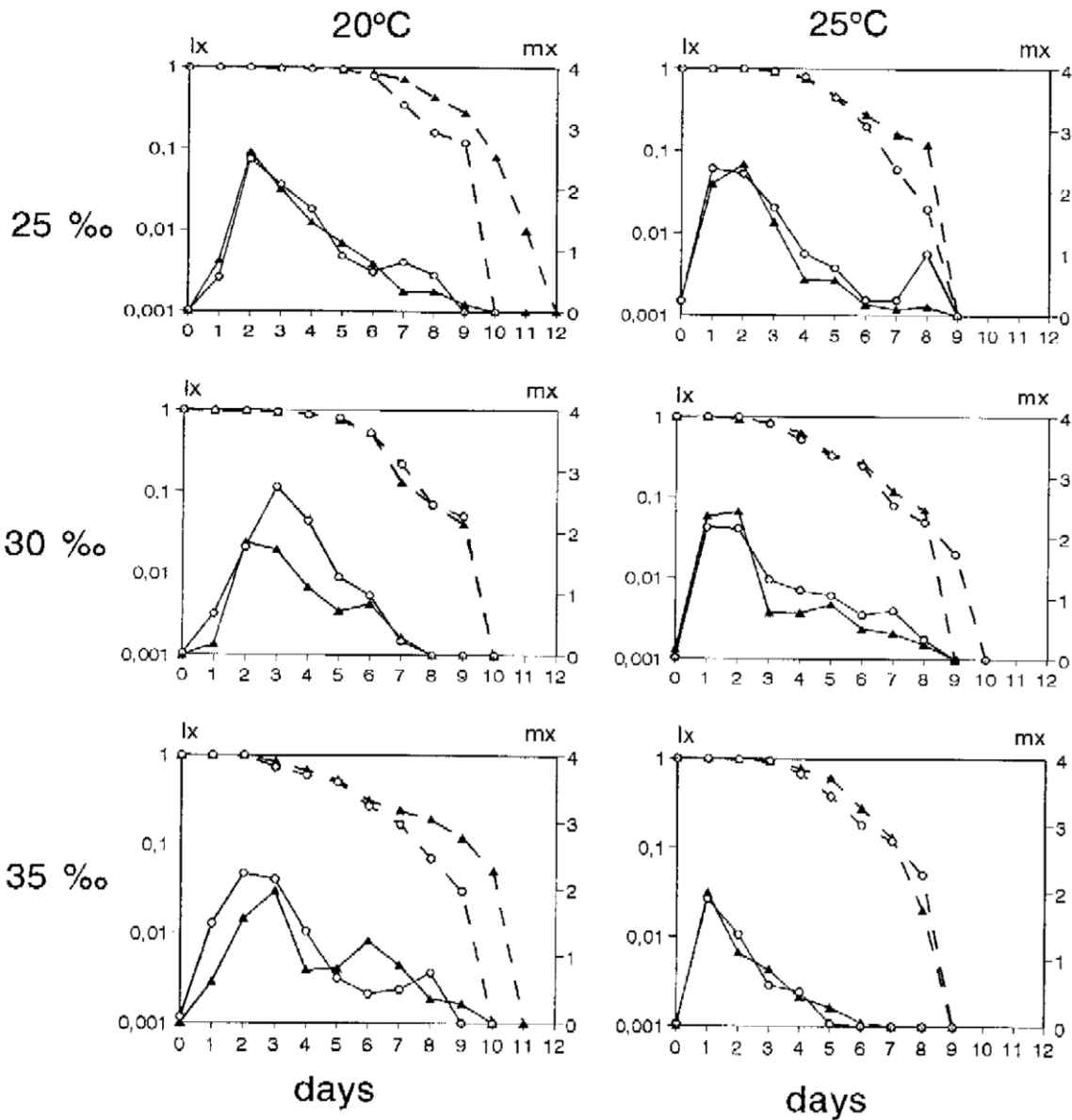


Figure 1.3.2 shows Age-specific survival (l_x) dashed line. and fecundity (m_x), newborns per female. for *S. littoralis* grown at temperatures of 20°C and 25°C, salinities of 25‰, 30‰ and 35‰. and food levels of *Tetraselmis* sp. 75,000 cells ml^{-1} (circles); 150,000 cells ml^{-1} (triangles). (BOSQUE ET AL. 2001)

As *S.c. valentine* is exposed to a wide range of temperatures (18-28°C) as well as salinities (18 - 36%) under natural conditions (OLTRA AND TODOLF 1997), one could expect that their optimum intrinsic growth rate will be in a range of their habitual conditions. It seems that ideal conditions for reproduction, generation time and intrinsic growth rate (defined as sum of survival, fecundity, timing of development and reproduction (MIRACLE AND SERRA 1989) decreased in the range of temperatures of 20 to 24°C and a salinity of 25‰.

Food concentration has to be higher at temperatures above the optimum, because the metabolic rate is higher (MIRACLE AND SERRA 1989). As a higher temperature imposes higher stress, it is not

surprising that lifespan is shorter in higher temperatures even with a higher food concentration. A similar effect of only two combined stressors, temperature and food, has been revealed for the calanoid copepod *Eurytemora affinis* by BAN (1994). *E. affinis* was caught in Lake Ohnuma in southern Hokkaido, Japan, and cultivated for at least three generations before the experiment. The copepods were reared under six food-temperature conditions (10^3 , 5×10^3 , 10^4 cells ml^{-1} at 15°C and 5×10^4 cells ml^{-1} at 10°C and 20°C). They were fed on a 1:1 (cell:cell) algae mixture of *Chlamydomonas* and *Cryptomonas tetrapyrenoidosa*. Temperature as well as food concentration had an influence on egg production and post-embryonic development time. Under low food provisioning, *E. affinis* produced less eggs and had a longer development period than individuals reared at a high food level and lower temperature. At the highest food level, females needed an average of 9, 2 days (20°C) from hatching to adult. At 10°C and the same food level, they needed 22, 8 days. Not only clutch size and development time, but also adult body size was influenced by temperature and food concentration. This is perceivable in female prosome length, which decreased about 10 % with increasing temperature (from 10°C to 20°C). Changes in food concentration from 5×10^4 to 10^3 cells ml^{-1} had a stronger impact on prosome length, as it decreased by 25%. This is not surprising, since prosome length and clutch size were positively correlated.

Another example on copepods affected by a combination of different stressors with temperature and food concentration is given by J. VIDAL (1980). He examined the effects of temperature, body size and food concentration on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. They were cultured at three temperatures (15.5° , 12° and 8°C) and several concentrations (0,3 ppm to 13,85 ppm) of a mixture of the diatoms *Thalassiosira eccentrica* and *T. angustii*. In general, individual body weight for both species increased sigmoidally with age. Individuals raised at low temperatures developed a higher body mass (measured in dry weight) than those cultured at higher temperatures. However, the influence of temperature in relation to the effect of food concentration on body mass was distinctly lower. This was also described before for *E. affinis* by BAN (1994).

VIDAL (1980) revealed differences in required food concentration at which the maximum body weight was observed. Required food concentration was substantially higher for the larger species (*C. pacificus*) than for the smaller *Pseudocalanus* species and increased with increasing body size as well as with increasing temperature. Due to this and since the critical food concentration for growth increased with body size, markedly more at higher than at lower temperatures, the author suggest that small copepod species (e.g. *C. pacificus*) are more influenced by temperature and large species more by food concentration. This gives an impression of adaption of those two copepods' life strategies to changes in temperature and food availability.

Obviously, Copepods have different growth or survival strategies during development (BAN 1994). In early stages, dry weight is comparatively unaffected by either temperature or food concentration. But in late stages, body mass was reciprocally to temperature and increased with food concentration. For example was there a significant relationship between temperature and body size for the stages CII to CIV of *C. pacificus*. The food concentration required for maximum body weight increased with stage of development. However, copepodites are able to optimize their growth independently of the required food concentration. For example was prosome length at a algae concentration of 10^4 and 5×10^3 cells ml^{-1} not significantly different to the length of well fed individual at a concentration 5×10^4 cells m^{-1} , at high temperatures. Under equal food conditions, late stages showed higher growth rates than at low temperatures.

LEVIN AND CREED (1986) found similar results for development of different larvae types of *Streblospio benedicti* (Polychaeta: Spionidae). They examined differences in reproduction and survival of planktotrophic (feeding on plankton) and lecithotrophic (feeding on yolk) larvae. Cultures of *S. benedicti* were reared under three temperature regimes, constant 20°C , mimicking natural conditions from November to July (temperature range from 7.5 to 27.8°C) and mimicking summer to fall and winter conditions from June to February (temperatures in a range of 7.5 to 30°C). Larvae of each temperature regime were held at two food levels (5 ml or 10 ml of a slurry with equal parts of sediment and seawater).

The authors described an increased reproduction and a significant higher survival rates ($p < 0.0001$) in adults with lecithotrophic development at the higher food level. No significant differences were observed in planktotrophic larvae's survival.

The only observed effect of a higher food level on larvae with planktotrophic development was an increase in adult length at constant and summer-fall temperature regimes. However, for all treatments considered together, LEVIN AND CREED (1986) did not find any significant differences in reproduction activity between the two development types. Temperatures simulating the summer-fall regime had an influence on reproduction activity of planktotrophic females ($p = 0.025$). No influences in lecithotrophic larvae and in winter-spring regimes were observed. As well as in the studies of OLTRA AND TODOLF (1997) and HALSBAND-LENK (2004), food concentration seemed to have a lower effect on reproduction than temperature.

Since literature about the influences of multiple stressors on vital rates of marine fish larvae is underrepresented, we refer to two different estuarine juvenile fish (MALLOY ET AL. 1991; PETERS AND BOYD 1972) to gives insight in conditions which are closely related to them in marine environments.

A good example for a stronger influence of at least two combined stressors is a study by PETERS AND BOYD (1972) on larvae of an estuarine flatfish. The Hogchocker, *Trinectes maculatus*, occurs in freshwater as well as in marine waters. studied juvenile fish in an age of about one year (31-39 mm on size). Juvenile fish were caught by trawl in the Newport River N.C., USA. In the experiment, the animals were exposed to combinations of temperatures of 15, 25 and 35°C, salinities of 0, 15 and 30‰ and fed on *Enchytraeus* sp., a terrestrial white worm, at three food levels, ad libitum (maximum unrestricted feeding), half ad libitum and no food. Growth rate and food conversion were measured.

Contrasting to the results of J. VIDAL (1980) and BAN (1994), both authors revealed a higher influence of food concentration than of temperature and food conversion in Hogchocker's larvae was influenced by temperature and salinity. Hence, the effect of food availability, considering ad libitum and half ad libitum feeding, seems less important. These findings are supported by the following results. The highest conversion to body tissue was reached at temperatures of 15° to 25°C and salinities of 15‰ to 30‰, with its maximum at 30‰. The lowest conversion for both food concentrations was reached at a temperature of 25°C combined with a salinity of 0‰ and it increased with salinity. As well the lowest temperature (15°C), as the highest (35°C), led to an increase in food conversion. Similar results were revealed by MALLOY AND TARGETT (1991). Among others, they measured growth rate for juvenile summer flounder (*Paralichthys dentatus*) caught near Roosevelt Inlet, Delaware, USA. Growth rate was measured at 2, 6, 10, 14, 18° C and 10, 20 and 30‰ salinity. Mean growth rate increased from 2, 4% d⁻¹ at 14° C to 3, 8 % d⁻¹ at 18°C and a slightly significant interaction between temperature and salinity (p = 0.043) was found. However, no independent salinity effect has been found. Growth rates in ad libitum feeding conditions showed similar results.

1.4 Response to Nutrition and Oxygen

Hypoxia in oceanic waters is mainly caused by a combination of abiotic factors as salinity, radiation and wind which leads to stratifications in the water column. As consequence of eutrophication in estuarine and coastal environments, hypoxia occurs regularly. Due to climate change and anthropogenic influences, hypoxic conditions are increasing. How hypoxia in multiple stressor systems affect marine Zooplankton is studied insufficiently. In consideration of a lack of studies on marine zooplankton, we chose the estuarine copepod, *Acartia tonsa*, to give an example of effects of hypoxia. *Acartia tonsa* is an important link between primary producers and consumers. The impact of hypoxia and food availability on the egg production of *Acartia tonsa* has been studied by SEDLACEK ET AL. (2005). In these experiments three different DO (dissolved oxygen) concentrations (0.53, 1.37 and 5.32 ml/l O₂) were combined with three food concentrations (600, 1200, and 1800 cells/ml of the dinoflagellate, *Akashiwo sanguinea*).

All experiments were conducted at a salinity of 30‰ and a temperature of 20°C. Figure 1.4.1 shows *A. tonsa* exposed to the different oxygen concentrations, egg production was lower at low DO concentrations (min. at 0.53-ml/l O₂) (SEDLACEK ET AL. 2005).

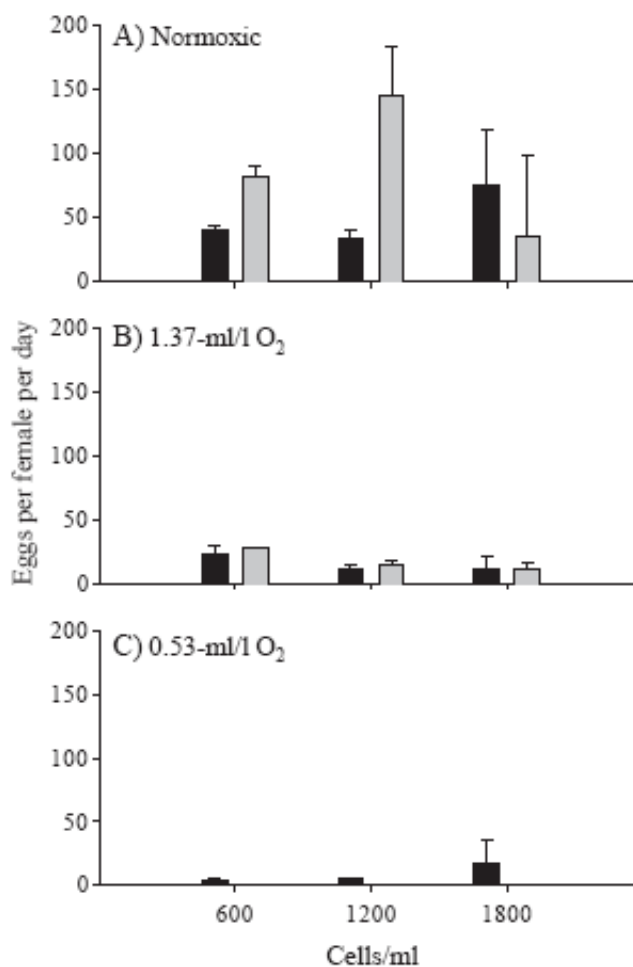


Figure. 1.4.1 shows mean egg production per female for *Acartia tonsa* (F1 S.D.) fed on different concentrations of *Akashiwo sanguinea* (GSBL) in cells/ml, on Day 1 (black bars) and Day 5 (gray), at different DO treatments: (A) normoxic control, (B) 1.37 ml/l O₂, and (C) 0.53 ml/l O₂. (SEDLACEK ET AL. 2005).

This was also shown in a study on survival and fecundity of *A. tonsa* by MARCUS ET AL. (2004). They described a high impact of hypoxia on both lethal and sublethal DO concentrations. Egg production in normal conditions was found to be significantly ($p=0.0404$) greater than at a DO concentration of 0.53 ml/l or 0.7 ml/l (referred to MARCUS ET AL. 2004).

During the second experiment, SELDACEK ET AL. 2005 exposed the copepods were to different DO concentrations as well as to different food concentrations. No weakening effect of a higher food concentration occurred. There seems not to be an evident general pattern due to reactions of *A. tonsa* on hypoxia combined with different food concentrations. In additional experiments,

RICHMOND ET AL. (2006) examined the effect of temperature and hypoxic conditions on *A. tonsa*. Survival and egg production were tested at normoxia (continually bubbled seawater) used as control, sublethal hypoxia (1.5 ml/l^{-1}) and lethal hypoxia (0.7 ml/l^{-1}) DO concentrations combined with two different temperatures simulating summer (25°C) and winter (15°C) conditions. As well lethal as sublethal conditions led to a slower development and maturity occurred at a smaller body size compared to individuals held under normal conditions. As an example, copepod stages in the control occurred on the third and fifth day at hypoxic conditions. Survival decreased significantly at lethal hypoxia conditions in comparison to both sublethal and controlled conditions. Not only development, maturity and survival were influenced by decreasing oxygen concentrations, but also egg production. In winter as well as in summer conditions, egg production was significantly lower at 0.7 ml/l^{-1} and at 1.5 ml/l^{-1} compared to normal conditions.

1.5 Response to Predation and food availability

As specified above, is zooplankton evidentially influenced by multiple stressors including food availability as treatment (e.g. DAGG 1977; OLTRA AND TODOLF 1997 SEDLACEK ET AL. 2005). In the case of a multiple stressor system, predation might be as important as nutrition and other stressors might have an influence on predation risk. Once prey behavior changes due to influences of treatments, this could lead to changes in prey availability. If for example jumping rates of *Acartia tonsa* are decreasing under the influence of high concentrations of dissolved oxygen, predation by ctenophores as *Mnemiopsis leidyi* is more likely (DECKER ET AL. 2004). A similar effect occurred when a high risk for predation was combined with high food availability. E.g., *A. tonsa* avoids patches of high food concentration when the risk of predation is too high (SAIZ ET AL. 1993). *A. tonsa* spent less time in food patches (fed on *T. weissflogii*) when exposed to predation by *Labidocera aestivu*.

Additionally increases predation and egg production: significantly more eggs were produced when the predator *Labidocera aestivu* was present ($P < 0.02$). Egg production of *A. tonsa* were dependent on food concentration and patchiness (Fig. 1.5.1) and egg production was highest in homogeneous distributed food at highest food concentration (SAIZ ET AL. 1993).

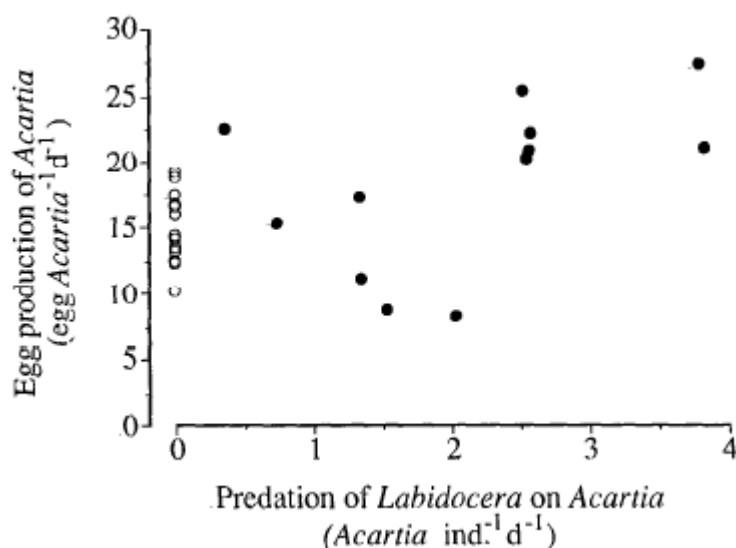


Figure. 1.5.1: shows egg production rate of *Acartia tonsa* under predation pressure of *Labidocera aestiva*. (solid circles) and without predation (hollow circles) (SAIZ ET AL. 1993).

The relation between egg production at homogeneous and heterogeneous food distribution was however the same. As described before, egg production in copepods depends on body size and ingestion rate (BAN 1994; GILLOOLY, ET AL.2001; KIØBOE ET AL. 1985). Therefore, it is not remarkable that egg production is increasing at higher food availability. If we have a look at the predators (*L. aestivu*) reproduction, twice as many eggs were produced when feeding on *A. tonsa*, then when feeding on algae. This is more likely to expect than an increase in egg production caused by predation, as *A. tonsa* showed in this experiment. As discussed by the authors, predators select the slower and healthier copepods, which would lead to a higher egg production by stronger, bigger and healthier individuals.

1.6 Response of Nutrition combined with Turbidity, Light intensity and turbulence

Availability of food in general is one of the most important requirements for life, beside oxygen. Since distribution has an impact on egg production (SAIZ ET AL. 1993), changes in food availability due to changes of environmental factors are becoming more important. Effects of stratification on Zooplankton are well-known. Predictions on changes in ocean circulation and climate induced variances of currents such as upwellings, are abundant (e. g. PISIAS 2001; BAKUN 1990; ROEMMICH AND MCGOWAN 1995). Influences of turbulence on other biotic or abiotic stressors are insufficiently explored. As an example of turbulence influencing organisms in a multi treatment system we will refer to a study of CHESNEY 1989.

In a laboratory experiment, larvae of striped brass (*Morone saxatilis*) were separately exposed to different turbidity values of 40, 90, 130 NTU (Nephelometric Turbidity Unit) produced by 50, 100, 150 ppm kaolin, turbulence and light intensities of (2000, 1000, 600, 300 lux), In a second

experiment, larvae were exposed to combinations of light intensity (450, 70, 12, 0.4 lux) in combination with turbidity (150ppm kaolin) and turbulence to examine if these variables mitigate food requirements (Fig. 1.6.1). Turbidity, light intensity and turbulence did have no significant effect on the survival rate, but a significant effect on growth of the larvae during the first 25 days after hatching was existent. CHESNEY 1989 showed that quantity of kaolin had no significant effect on the growth rate. Influences on growth in experiments with combinations of the variables, showed enhanced effects of turbulence by adding turbidity, for example did larvae grow faster when turbidity was combined with turbulence than in turbulence alone ($p = 0.05$). On the other hand does light reduction or addition of turbulence show a significant reduction in growth and foraging rates of the fish larvae. Light intensity alone is compared to effects of an exposure combination not decisively affecting growth and survival of striped bass larvae. Even at very low light intensities and up to completely darkness the bass larvae were able to grow and survive. Despite the effect of light is not to be neglected. As an example growth rates at a low light level of 600 lux vary between $0.28 \text{ (mm d}^{-1}\text{)}$ and $0.39 \text{ (mm d}^{-1}\text{)}$ at 2000 lux in comparison to the light-turbidity-turbulence treatment ($0.20 \text{ (mm d}^{-1}\text{)}$ at 450 lux and 150 ppm kaolin) (CHESNEY 1989).

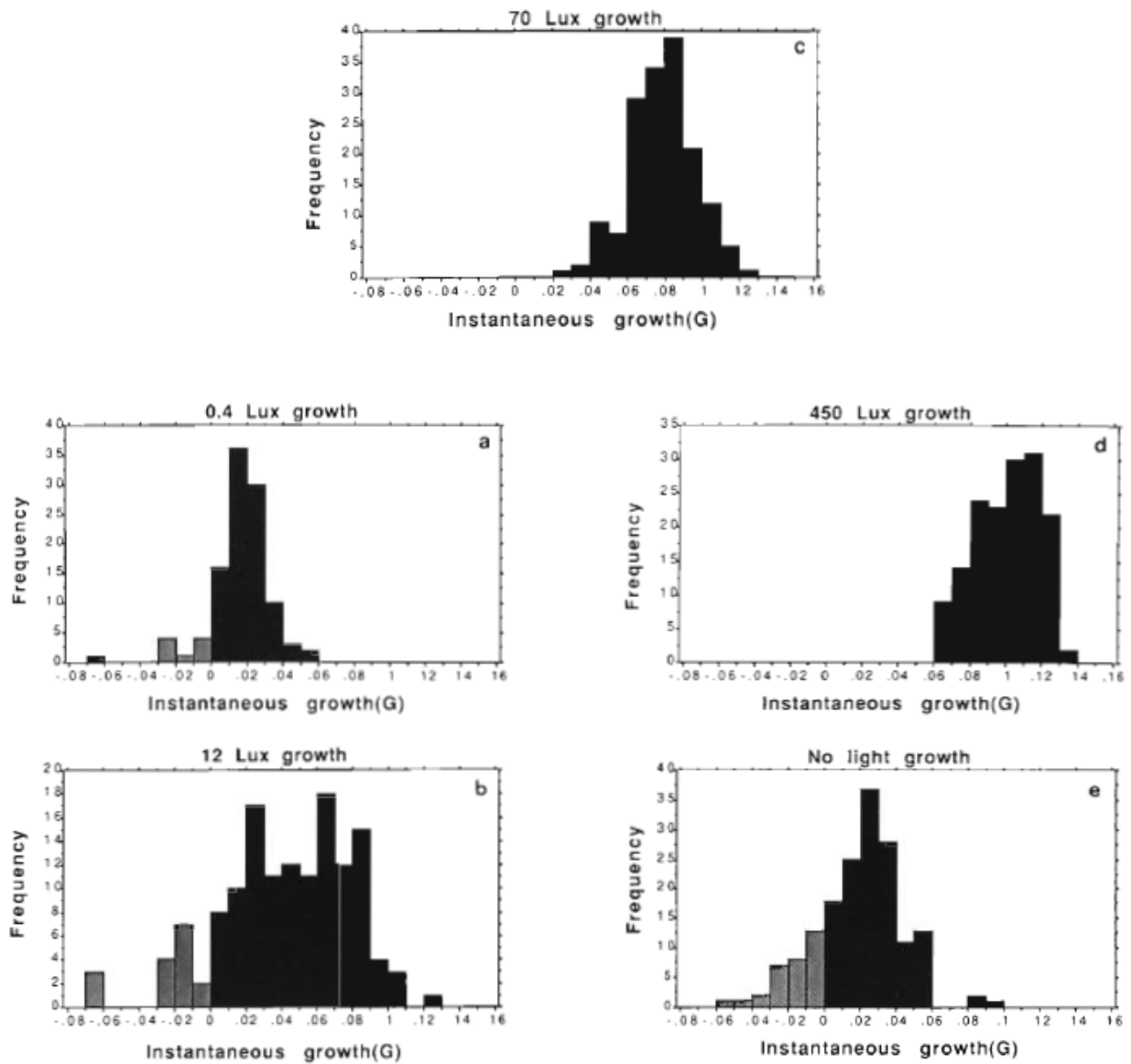


Figure 1.6.1 shows. frequencies of individual daily instantaneous growth coefficients (G) of striped bass larvae (*Morone saxatilis*) at 0.4, 12, 70 and 450 lux with turbulence and turbidity and in total darkness without turbulence or turbidity. Shading contrasts positive and negative growth (CHESNEY 1989).

As CHESNEY 1989 examined in his experiments is turbidity influencing marine organisms directly, as well as it is influencing other stressors (e.g. turbulence). In despite that climate change might induce changes in sediment flow and increased input of coastal soil, the impact of turbidity is getting more important. Combined effects, whether moderating or intensifying, are studied insufficiently. Except for of the study above, there are some studies on other organisms than zooplankton in e.g. on an adult planktivorous fish (*Gobiusculus flavescens*) by UTNE 1997 and one describing the effects of turbidity, light and food concentration on juvenile weakfish (*Cynoscion regalis*) (GRECAY AND TARGETT 1996).

Weakfish were exposed to turbidities ranging from 0.95 to 11 NTU, light levels from dark to $>0.01 \cdot 10^{14}$ quanta $s^{-1} cm^{-1}$ and fed on mysid shrimps (*Neomysis americana*). As investigated by CHESNEY ET AL 1989 did increasing light level enhance survival and growth rates? Whether turbidity values are high or low; foraging success is dependent on adequate food concentration respective to food availability. Complete darkness did reduce feeding at food concentrations of 0.5 to 4 times the typical density of Delaware Bay (400 mysids m^{-3}). This is assumed to be caused by a decreased foraging efficiency.

1.7 Conclusion and future response

This review gives an overview of recent studies on the influences of natural multiple stressors on the vital rates of marine zooplankton organisms. Due to a lack of literature it is an onerous task to give an overview on a broad spectrum of marine zooplankton organisms affected by combined natural stressors. Therefore, we included a few examples on estuarine organisms. The main organisms studied are copepods, since they are one of the most important links in the food web. A few studies of other zooplankton organisms or planktonic developmental stages, e.g. polychaetes, sea urchin larvae and rotifers, are included. The most often revealed natural stressors are temperature and salinity, but some studies about turbidity, hypoxia and food availability have also been published and are included in this review.

To sum up, there are large differences in vital rates of development types, if single stressors are considered. This has been revealed by LEVIN AND CREED (1986). But a view on a combination of all tested treatments also shows high significant interactions of combined stressors (e.g. BAN 1994; HOLSTE ET AL. 2009; SERRA ET AL. 1994) as no significant differences on stressors affecting vital rates (e.g. HALSBAND-LENK 2004; LEVIN AND CREED 1986 OLTRA AND TODOLF 1997; SEDLACEK ET AL 2005). Apparently, it is not possible to give any general statement on the influence of multiple stressors on vital rates of marine zooplankton organisms. The intensity of multiple stressors compared to single stressors is clearly dependent on a wide range of evolutionary and environmental factors. Different populations (DEVERKER ET AL. 2007), as well as different species (BOSQUE ET AL. 2001; OLTRA AND TODOLF 1997) differ in development rates. Since some organisms are able to adapt to changes in environmental conditions very quickly, e.g. *Eurytemora affinis* (Deverker 2007), these are less affected by changes of a single stressor. However, influences of multiple stressors might be antagonistic, even well adapted populations can be depleted (HARLEY ET AL. 2006, FOLT ET AL. 1999).

Acclimation and adaption of early zooplankton stages play a decisive role concerning mortality and survival (e.g. DAMGAARD AND DAVENPORT 1994; NAGARAJ 1988) as well as reproduction (HOLSTE ET AL. 2009) and development (NAGARAJ 1988). A positive effect of acclimation was examined by HOLSTE ET AL. (2009). They observed a higher tolerance of salinity when copepods were adapted

to high values of combinations of salinity and temperature. It seems as if tolerance of various combinations depends on the taxa which is exposed to the stressors and on the stressors themselves (e.g. BAN 1994; BORCHERT ET AL. 1996).

Moreover the reaction of an organism which is exposed to multiple stressors, is dependent on biological factors, such as age respective developmental stage (NAGARAJ 1988), body size and mass (GILLOOLY et al. 2001 and 2002), as well as individual physical condition. Hence, nutritional state has an impact on the sensitivity of an organism. (e.g. HEUGENS 2001).

Further research on natural multiple stressors affecting zooplankton is needed to take into account all factors described above. It is important to understand exactly how environmental changes are influencing organisms in different trophic levels. As far as we know how these stressors operate on different organisms in the marine food web, we can make more precise predictions concerning impacts of toxins and other treatments on different marine taxa. Since plankton is the main food source in the aquatic environment, there is a high need for research on a broader spectrum of zooplankton organisms to obtain a better insight as to the impacts of multiple stressors in marine environments.

2. Statistical analyses of experimental activities

Full details of the experiment and results are provided in D1.6

2.1 Temperature and the Herbicide (Terbuthylazine): phytoplankton interactions (UNIBO)

Modelling parameterisation

Given the known effect of the triazine herbicides on Photosystem II the parameterisation under development acts on the photosynthesis process.

The algorithm developed is:

$$\frac{\partial P_c}{\partial t} = \left(\frac{\partial P_c}{\partial t} \Big|_{gpp} - \frac{\partial P_c}{\partial t} \Big|_{loss} \right) * \left(1 - \frac{C}{C_{inib}} \right)$$

Where C is the herbicide concentration and C_{inib} is the herbicide concentration value for which that inhibits completely the photosynthesis.

2.2 Copper, oxytetracycline: Protozoan *Euplotes crassus* (UPiedmont)

2.2.1 Protozoan Survival relative to Copper and temperature exposure.

The survival of *E. crassus* exposed to copper as illustrated in figure 2.2.2 shows a tendency to a higher survival at the lowest temperatures examined over the whole range of copper concentrations, although when comparing the curves statistically there is no significant difference between the curves for survival ($P>0.05$). All parameters for the equations are in table 2.2.1

Table 2.2.1 Estimated LC_{50} values and confidence limits applying the EPA Probit Analysis Program (Version 1.5) to data obtained exposing protozoa to copper and temperature.

Temp.	LC_{50} (mM)	95% Confidence Limits	
		Lower	Upper
25°C	12.950	8.403	20.126
27°C	9.095	5.429	15.468
29°C	6.263	3.320	11.912
31°C	5.570	2.943	10.448
33°C	4.485	2.345	8.230

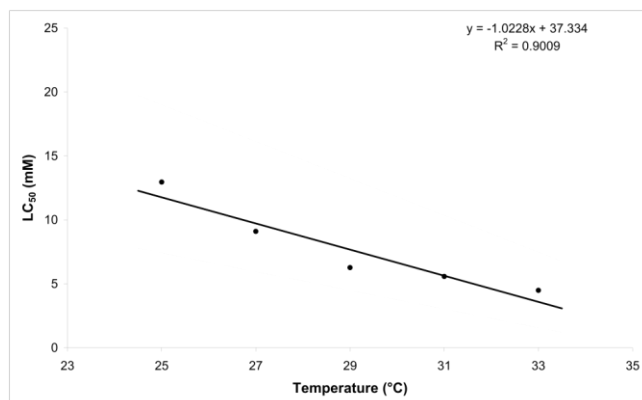


Figure 2.2.1 Relationship between LC_{50} and temperature estimated analysing ecotoxicological data on protozoa exposed to copper.

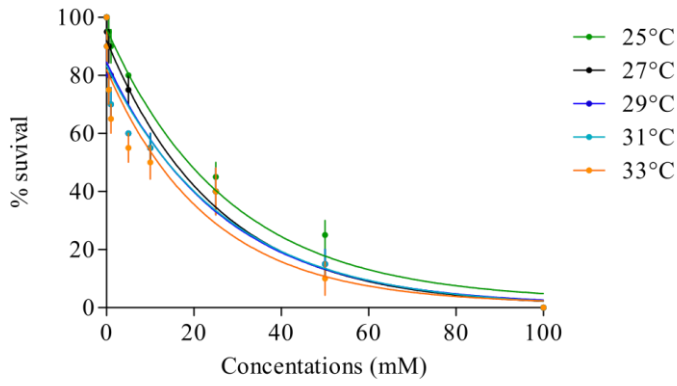


Figure 2.2.2 The percentage of survival when exposed to copper at different temperatures.

2.2.2 Protozoan replication exposed to variations on copper and temperature

The replication of *E. crassus* exposed to copper as presented shows a tendency for a higher sensitivity to copper at the high temperatures, although when comparing the curves statistically there is no significant difference between the curves for replication ($P > 0.05$).

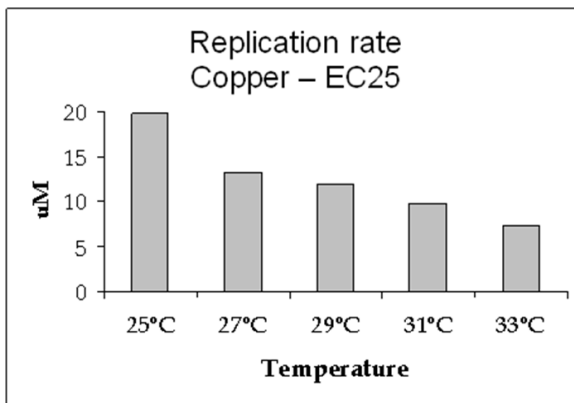


Figure 2.2.2.1 Toxicological effects of copper to the protozoan *Euplotes crassus*: replication rate, as EC_{25} .

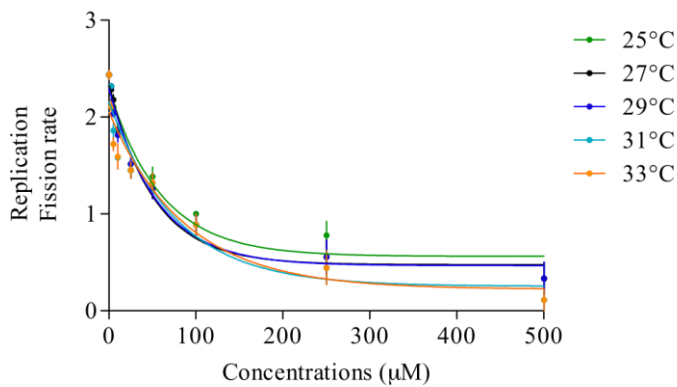


Figure 2.2.2.2 The protozoan replication when exposed to copper at different temperatures.

The results were fitted to the equation; one phase association found in GraphPad Prism 5:

$$Y = (Y_0 - \text{Plateau}) * \exp(-K * x) + \text{Plateau}$$

The Plateau is the Y-value when the curves have stabilized, Y₀ is when the line hit the y-axis and K is the rate constant. To compare the equations an extra sum of squares analysis for nested models in nonlinear regression was used to find eventual difference in curves (McCullagh and Nelder 1989). Furthermore, relevant single parameters in the equations were compared separately using student T-tests.

Table 2. Parameters for the equation: one phase association found in GraphPad Prism 5. For the protozoan *Euplotes crassus* exposed to copper at different temperatures.

		Best fit values (95% confidence intervals)		
		Y ₀	Plateau	K
<i>Euplotes crassus</i> Survival	25°C	95.98 (91.38-100.6)	2.277 (-8.874-13.43)	0.03595 (0.02413-0.04776)
	27°C	91.98 (87.55-96.41)	0.4449 (-9.378-10.27)	0.03942 (0.02730-0.05155)
	29°C	84.51 (78.62-90.39)	0.7639 (-12.71-14.24)	0.03805 (0.02075-0.05535)
	31°C	83.32 (77.14-89.49)	0.1834 (-14.49-14.85)	0.03662 (0.01864-0.05460)
	33°C	81.89 (74.88-89.07)	1.217 (-13.47-15.90)	0.04267 (0.01974-0.06561)
Reproduction	25°C	2.288 (2.171-2.405)	0.5622 (0.4137-0.7107)	0.01663 (0.01168-0.02159)
	27°C	2.325 (2.196-2.455)	0.4765 (0.3224-0.6305)	0.01919 (0.01348-0.02489)
	29°C	2.288 (2.162-2.414)	0.4671 (0.3135-0.6207)	0.01813 (0.01274-0.02352)
	31°C	2.152 (2.024-2.279)	0.2540 (0.07051-0.4376)	0.01304 (0.008878-0.01721)
	33°C	2.050 (1.917-2.183)	0.2231 (0.01535-0.4308)	0.002086 (0.007224-0.01554)

2.3 Temperature and 4-n-nonylphenol: Copepod *Acartia tonsa* (SDU, UHAM)

The equation generated by an earlier study is to calculate egg production, hatch success and survival for *Acartia tonsa* in our experiment. The equation is as follows:

$$P(T) = P_{\text{opt}} * ((T_{\text{max}} - T) / (T_{\text{max}} - T_{\text{opt}}))^a * \exp(a * (T - T_{\text{opt}}) / (T_{\text{max}} - T_{\text{opt}}))$$

As adult copepods die at 34°C (Holste and Peck 2006) the maximal temperature (T_{max}) was set at 34°C for our calculations. The other variables employed i.e. max production/survival of copepods (P_{opt}±se), optimal temperature (T_{opt}±se) and the factor determining the shape of the curve (a±se) are shown in table 1 together with the 95% confidence intervals.

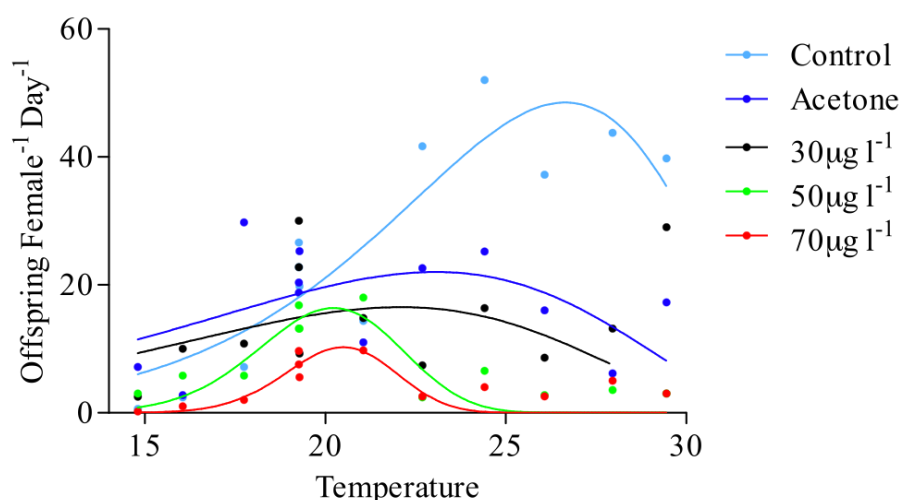


Figure 2.3.1 Significant relationship: offspring survival after seven days of exposure. Acetone: acetone control $100\mu\text{l l}^{-1}$.

Table 1. Best fit values and 95% confidence intervals found in GraphPad Prism 5.

		Control	Acetone	$30\mu\text{g l}^{-1}$	$50\mu\text{g l}^{-1}$	$70\mu\text{g l}^{-1}$
Egg production	Popt	63.97 (49.48-78.47)	58.79 (35.66-81.93)	45.57 (29.62-61.52)	Data points do not fit the equation	66.44 (49.00-83.88)
	Topt	27.19 (22.96-31.41)	26.43 (20.83-32.03)	25.89 (21.04-30.75)		28.35 (21.27-35.44)
	a	0.97 (-0.8504-2.791)	1.21 (-1.988-4.401)	1.19 (-1.616-4.004)		0.65 (-1.259-2.565)
Hatch success	Popt	89.83 (69.07-110.6)	43.93 (28.37-59.50)	76.31 (48.45-104.2)	49.40 (23.13-75.66)	47.61 (34.83-60.40)
	Topt	25.00 (24.18-25.82)	23.21 (21.60-24.82)	25.07 (23.90-26.25)	24.98 (23.15-26.80)	25.17 (23.64-26.70)
	a	7.53 (3.653-11.40)	7.92 (0.501-15.33)	9.39 (2.120-16.65)	8.25 (-1.337-17.84)	3.45 (0.544-6.357)
Survival of offspring	Popt	48.51 (37.27-59.74)	22.01 (12.33-31.70)	16.52 (7.149-25.90)	16.39 (10.84-21.94)	10.26 (4.792-15.73)
	Topt	26.65 (25.17-28.13)	23.06 (19.88-26.25)	22.09 (17.72-26.46)	20.23 (19.33-21.12)	20.50 (19.66-21.33)
	a	3.19 (0.631-5.757)	3.39 (-1.903-8.684)	4.24 (-5.542-14.02)	48.26 (2.460-94.05)	78.42 (-25.95-182.8)

To examine development seven day old offspring were divided into nauplii stages and copepodite 1 to adult (C1-C6). For the % C1-C6 there was a sigmoid increase towards higher temperatures. The Boltzmann sigmoid equation found in GraphPad Prism 5, was fitted with the minimum percentage (Pmin) and maximum percentage (Pmax) defined as 0 and 100% respectively. The temperature where 50% have developed to another stage (T50) and the slope of the curves (Slope) are shown in table 6 together with the 95% confidence intervals.

Boltzmann sigmoid equation as follows

$$P(T) = P_{\min} + ((P_{\max} - P_{\min}) / (1 + \exp(T50 - T / \text{Slope})))$$

Comparing the curves for the developmental stages there were significant differences ($P < 0.01$) and the T50 values of the treatments at concentrations of $50\mu\text{g l}^{-1}$ and $70\mu\text{g l}^{-1}$ nonylphenol relative to the control ($P < 0.01$). Hence, there was a delay in the development in the $50\mu\text{g l}^{-1}$ and $70\mu\text{g l}^{-1}$ treatments. Similarly was a significant delay for replicates at 19.3°C there at the $50\mu\text{g l}^{-1}$ and $70\mu\text{g l}^{-1}$ treatments ($P = 0.004$ and $P < 0.0001$). It was not possible to find any difference in developmental

stages in temperatures above 22°C as almost all copepods were developed to adults after the seven days of exposure.

The best fit values for the curve and the 95% confidence intervals are shown in table 2.3.1

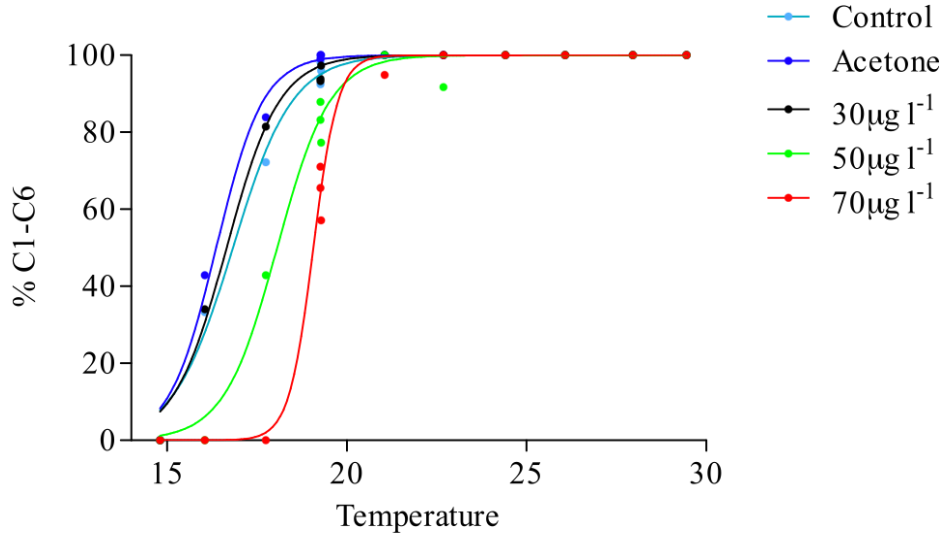


Figure 2.3.2. Stage of development after seven days of exposure. % copepodite 1- adult (C1-C6) of all survived offspring.

Table 2.3.1 Best fit values and 95% confidence intervals found in GraphPad Prism 5 for the equation Boltzmann sigmoidal.

		Control	Acetone	30µg l ⁻¹	50µg l ⁻¹	70µg l ⁻¹
% C1-C6	T50	16.83 (16.62-17.04)	16.37 (16.17-16.56)	16.65 (16.47-16.84)	18.06 (17.84-18.28)	19.07 (18.87-19.27)
	Slope	0.8268 (0.6731-0.9804)	0.6520 (0.4816-0.8224)	0.7370 (0.6015-0.8725)	0.7370 (0.5629-0.9111)	0.3410 (0.02269-0.6593)

2.4 Copper, nickel, oxytetracycline: mussels *Mytilus galloprovincialis* (UPiedmont) lysosomal membrane stability

Previous studies have described a clear correlation among lysosomal membrane stability and other high level ecotoxicological endpoints, such as scope for growth (Bayne et al 1979, Allen & Moore 2004). As known, the parameter scope for growth is related to the ability of a mussel population to growth, increasing the dimension of individuals and their number. The parameterization of the relationship between lysosomal membrane stability and scope for growth can allow linking alteration in the studied biomarkers (i.e. lysosomal membrane stability) with potential effects at organism and population level, applying the equation proposed by Allen & Moore (2004) (figure 3). Scope for growth (SFG) can be obtained from lysosomal membrane stability (LMS) applying the following equation:

$$SFG = 17.007 \times LMS - 260,850$$

Where

SFG is expressed in joules g⁻¹ day⁻¹

LMS is expressed in minutes

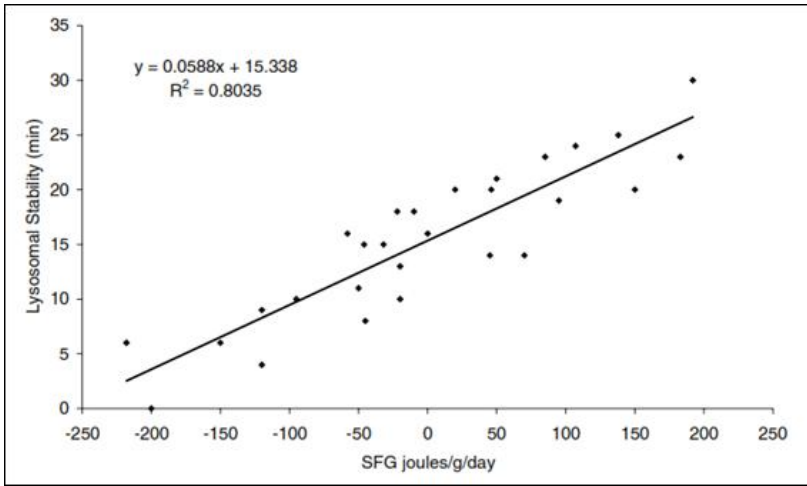


Figure 2.4.1 Relationship between lysosomal membrane stability (min) and scope for growth (joules $g^{-1} day^{-1}$) (from Allen & Moore 2004).

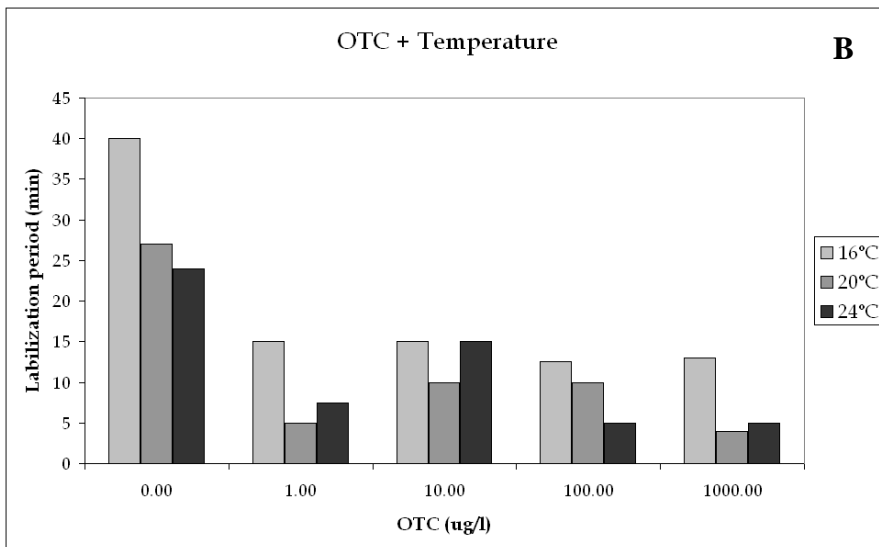
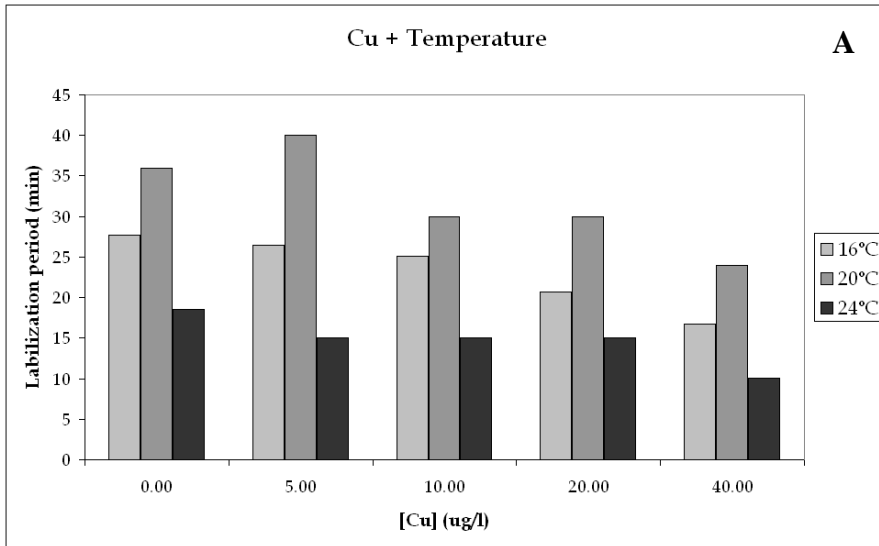


Figure 2.4.2. Results on lysosomal membrane stability in mussels exposed to Cu (panel A) and OTC (panel B) at different concentration and temperatures.

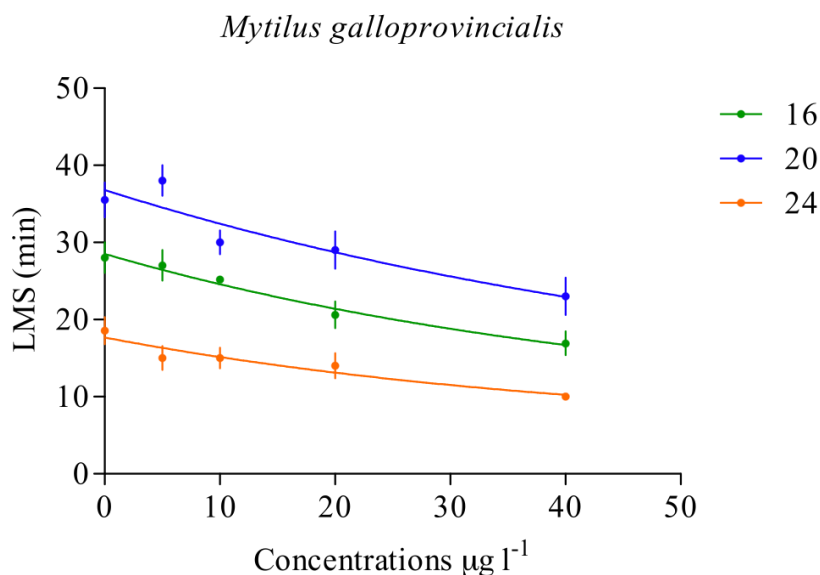


Figure 2.4.3. Lysosomal membrane stability (LMS) in marine mussels (*Mytilus galloprovincialis*) when exposed to copper at different temperatures.

The results were fitted to the equation; one phase association found in GraphPad Prism 5:

$$Y = (Y_0 - \text{Plateau}) * \exp(-K * x) + \text{Plateau}$$

The Plateau is the Y-value when the curves have stabilized, Y0 is when the line hit the y-axis and K is the rate constant. To compare the equations an extra sum of squares analysis for nested models in nonlinear regression was used to find eventual difference in curves (McCullagh and Nelder 1989). Furthermore, relevant single parameters in the equations were compared separately using student T-tests.

Best fit values and 95% confidence intervals found in GraphPad Prism 5.

	Best fit values (95% confidence intervals)		
	Y0	Plateau	K
<i>Mytilus galloprovincialis</i> 16°C	28.52 (25.60-31.45)	7.541 (-34.99-50.07)	0.02071 (0.0-0.08316)
LMS 20°C	36.82 (32.69-40.96)	8.336 (-84.79-101.5)	0.01669 (0.0-0.09228)
24°C	17.69 (15.08-20.29)	5.264 (-25.81-36.33)	0.02287 (0.0-0.1112)

2.5 Temperature and Copper: Herring *Clupea harengus membras* (SDU, UHAM)

Two parameters, % of viable larvae and larvae length an effect was observed. Those two parameters are presented here.

2.5.1 % Viable Larvae in relation to copper and temperature exposure.

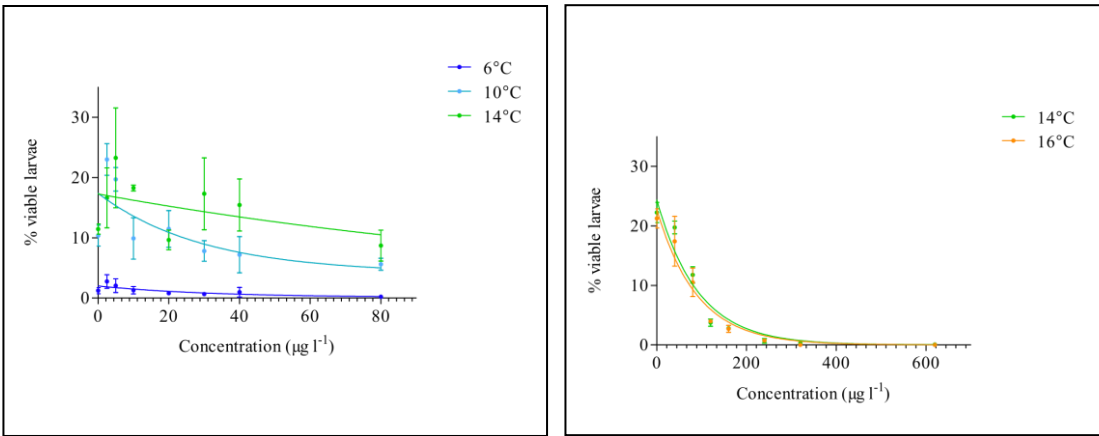


Figure 2.5.1. % viable larvae of the total larvae hatched for experiment 1 and 2. Error bars show the standard errors from three replicates. The lines on the graphs are one phase decay found in GraphPad Prism 5. Best fit values and 95% confidence intervals are shown in table 2.5.1.

Viable larvae from experiment 1 and 2: Best fit values and the 95% confidence intervals for the equation: one phase decay. The results were fitted to the equation; one phase association found in GraphPad Prism 5:

$$Y = (Y_0 - \text{Plateau}) * \exp(-K * x) + \text{Plateau}$$

The Plateau is the Y-value when the curves have stabilized, Y₀ is when the line hit the y-axis and K is the rate constant. To compare the equations an extra sum of squares analysis for nested models in nonlinear regression was used to find eventual difference in curves (McCullagh and Nelder 1989). Furthermore, relevant single parameters in the equations were compared separately using student T-tests.

Table 2.5.1 Best fit values and 95% confidence intervals

Best fit values (95% confidence intervals)		
	Y ₀	K
Experiment 1		
6°C	2.005 (1.128-2.881)	0.02851 (0.0-0.06325)
10°C	16.88 (12.90-20.85)	0.01936 (0.004965-0.03375)
14°C	17.28 (12.41-22.14)	0.006240 (0.0-0.01686)
Experiment 2		
14°C	24.37 (21.56-27.18)	0.0192 (0.008546-0.01329)
16°C	22.77 (19.34-26.20)	0.1116 (0.007988-0.01434)

2.5.2 Larval length vs temperature and copper

A key metric attributing to the competitive ability of larvae and their ability to escape predators is larval size (Litvak and Leggett 1992). In this experiment temperature had an effect on larvae length (figure 2.5.1) with control larvae reared at low temperatures (6, 10°C) being longer (0.6-0.8mm) than those reared at (14, 16°C). In the first experiment copper had an effect at larvae length at 6 and 10°C, with a decrease of 1.2 and 1.1mm respectively at 80µg l⁻¹ while at 14°C copper did not have an effect on larvae length. Best fit values and 95% confidence intervals from the exponential equation are shown in the table below

In the second experiment, copper had an effect at both 14 and 16°C at high concentrations, with an exponential decrease in length of 0.8 and 0.4mm at 80µg l⁻¹ while at copper concentrations at 160µg l⁻¹ the difference was at 1.0 and 1.2mm respectively. Best fit values and 95% confidence intervals are shown in table 8. Based on these results there is a trend to higher impact of copper at the low temperatures.

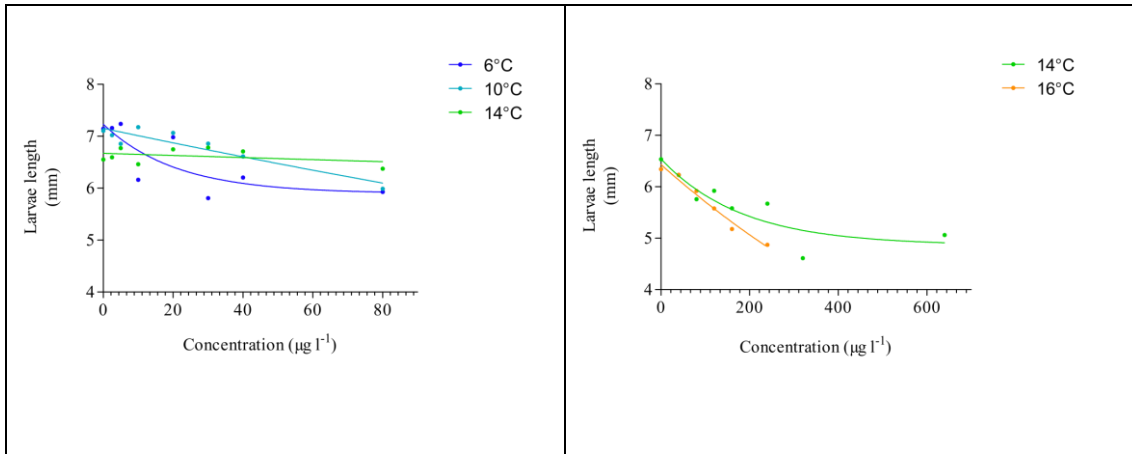


Figure 2.5.1 Length of newly hatched larvae from experiment 1 and 2. The equation of the curves are one phase decay. Best fit values and 95% confidence intervals are shown in table 2.5.2.

The results were fitted to the equation; one phase association found in GraphPad Prism 5:

$$Y = (Y_0 - \text{Plateau}) * \exp(-K * x) + \text{Plateau}$$

The Plateau is the Y-value when the curves have stabilized, Y₀ is when the line hit the y-axis and K is the rate constant. To compare the equations an extra sum of squares analysis for nested models in nonlinear regression was used to find eventual difference in curves (McCullagh and Nelder 1989). Furthermore, relevant single parameters in the equations were compared separately using student T-tests.

Table 2.5.2 Newly hatched larvae length; best fit values and 95% confidence intervals.

	Best fit values (95% confidence intervals)		
	Y0	K	Plateau
Experiment 1			
6°C	7.233 (6.424-8.042)	0.04794 (0.0-0.1732)	5.896 (4.651-7.142)
10°C	7.153 (6.883-7.423)	0.001992 (0.0-0.01657)	1.250*10 ⁻⁰⁰⁹ (0.0-48.18)
14°C	6.671 (6.460-6.883)	0.0003051 (0.0-0.02756)	1.536*10 ⁻⁰⁰⁸ (0.0-589.5)
Experiment 2			
14°C	6.542 (5.845-7.239)	0.005483 (0.0-0.1385)	4.862 (3.874-5.849)
16°C	6.431 (6.136-6.725)	0.001193 (0.0-0.007083)	8.345*10 ⁻⁰¹⁰ (0.0-27.56)

3. References

Alderdice DF (1972). Responses of marine poikilotherms to environmental factors acting in concert. In: O. Kinne (ed.), *Marine ecology*, vol. 1. Wiley, London: 1659-1722.

Anil AC, Chiba K, Okamoto K, Kurokura H (1995). Influence of temperature and salinity on larval development of *Balanus amphitrite*: implications in fouling ecology. *Marine Ecology Progress Series* 118: 159-166

Ban S (1994). Effect of temperature and food concentration on post-embryonic development, egg production and adult body size of calanoid copepod *Eurytemora affinis*, *Journal of Plankton Research* 16: 721-735

Bakun A (1990). Global climate change and intensification of coastal ocean upwelling. *Science*, 247: 198–201.

Bochert R, Fritzsche D and Burckhardt R (1996). Influence of salinity and temperature on growth and survival of the planktonic larvae of *Marenzelleria viridis* (Polychaeta, Spionidae). *Journal of Plankton Research* 18 (7): 1239-1251.

Bosque T, Hernández R, Pérez R, Todolí R, Oltra R (2001). Effects of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. *Journal of Experimental Marine Biology and Ecology* 258: 55–64.

Bryant V, Newbery D, M, Mc Lusky DS, Campbell R (1985). Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (*Corophium volutator*, *Macoma balthica*). *Marine Ecology Progress Series* 24: 139-153.

Chesney EJ Jr. (1989). Estimating the food requirements of striped bass larvae *Morone saxatilis*: effects of light, turbidity and turbulence. *Marine Ecology Progress Series* 53: 191-200

Crisp DJ and Costlow JD (1963). The tolerance of developing embryos to salinity and temperature. *Oikos* 14 (1): 22-34

Dagg M (1977). Some effects of patchy food environments on copepods. *Limnology and Oceanography* 22 (1): 99-107

Damgaard RM and Davenport J (1994). Salinity tolerance, salinity preference and temperature tolerance in the high-shore Harpacticoid copepod *Tigriopus brevicornis*. *Marine Biology* 118, 443-449

- Decker MB, Breitburg DL, Purcell JE (2004). Effects of low dissolved oxygen on zooplankton predation by the ctenophore *Mnemiopsis leidyi*. *Marine Ecology Progress Series* 280: 163–172.
- Fabry VJ, Seibel BA, Feely RA and Orr JC (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science*, 65: 414–43
- Folt CL, Chen CY, Moore MV and Burnaford J (1999). Synergism and antagonism among multiple stressors. *Limnol. Oceanogr*, 44(3): 864–877
- Frost BW (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod, *Calanus pacificus*. *Limnology and Oceanography*, 17: 805-815.
- Gaudy R, Cervetto G, Pagano M (2000). Comparison of the metabolism of *Acartia clausi* and *A. tonsa*: influence of temperature and salinity. *Journal of Experimental Marine Biology and Ecology* 247: 51–65
- Gillooly JF, Charnov EL, West GB et al. (2002). Effects of size and temperature on developmental time. *Nature*, 417, 70–73
- van Hal R, Smits K and Rijnsdorp AD (2010). How climate warming impacts distribution and abundance of two small flatfish species in the North Sea. *Journal of Sea Research*. 64 (1-2): 76-84
- Halsband C, Hirche H-J (2001). Reproductive cycles of dominant calanoid copepods in the North Sea. *Marine Ecology Progress Series*, 209:219– 229.
- Halsband-Lenk C, Hirche H-J, Carlotti F (2002). Temperature effect on reproduction and development of congener copepod populations. *Journal of Experimental Marine Biology and Ecology* 271:121–153.
- Halsband-Lenk C, Carlotti F and Greve W (2004). Life-history strategies of calanoid congeners under two different climate regimes: a comparison. *ICES Journal of Marine Science* 61: 709–720.
- Harley CDG, Hughes AR, Hultgre KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006). The impacts of climate change in coastal marine systems. *Ecological Letters*, 9: 228–241.
- Heugens EHW, Hendriks AJ, Dekker T, van Straalen NM and Admiraal Wim (2001). A Review of the Effects of Multiple Stressors on Aquatic Organisms and Analysis of Uncertainty Factors for Use in Risk Assessment. *Critical Reviews in Toxicology*, 31(3):247–284.
- Holste L and Peck MA (2006). The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation, *Marine Biology* 148: 1061–1070
- Holste L, St. John MA and Peck MA (2009). The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation. *Marine Biology*, 156: 527–540
- Kinne O (1960). Growth, Food Intake, and Food Conversion in a Euryplastic Fish Exposed to Different Temperatures and Salinities. *Physiological Zoology*, 33 (4): 288-317
- Kinne O, (1970). Temperature: Animals-Invertebrates. In: O. Kinne (ed.), *Marine ecology*, vol. 1. Wiley, London: 407-514.

- Kjørboe T, Mohlenberg F, Hamburger K (1985). Bioenergetics of the planktonic copepod *Acartia tonsa*, relation between feeding, egg production and respiration, and composition of specific dynamic action. *Marine Ecology Progress Series* 26: 85–97.
- Lee CE, Remfert JL and Gelembuik GW (2003). Evolution of Physiological Tolerance and Performance During Freshwater Invasions. *Integrative and Comparative Biology* 43:439–449
- Levin LA and Creed EL (1986). Effect of temperature and food availability on reproductive responses of *Streblospio benedicti* (Polychaeta: Spionidae) with planktotrophic or lecithotrophic development. *Marine Biology* 92, 103-113
- Malloy KD and Targett TE (1991). Feeding, growth and survival of juvenile summer flounder *Paralichthys dentatus*: experimental analysis of the effects of temperature and salinity. *Marine Ecology Progress Series* 72: 213-223
- Marcus NH, Richmond C, Sedlacek C, Miller GA and Oppert C (2004). Impact of hypoxia on the survival, egg production and population dynamics of *Acartia tonsa* Dana. *Journal of Experimental Marine Biology and Ecology*, 301 (2):111-128
- Miracle, M. R. and Serra, M. (1989) Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia*, 186-187: 81-102
- Möllmann C, Kornilovs G, Sidrevics L (2000). Long-term dynamics of main mesozooplankton species in the central Baltic Sea. *Journal of Plankton Research*, 22: 2015–2038
- Nagaraj M (1988). Combined effects of temperature and salinity on the complete development of *Eurytemora velox* (Crustacea: Calanoidea). *Marine Biology*, 99: 353 -358.
- Nelson GC, Bennet E, Berhe AA, Cassman K, DeFries R, Dietz T, Dobermann A, Dobson A, Janetos A, Levy M, Marco D, Nakicenovic N, O'Neill B, Norgaard R, Petschel-Held G, Ojima D, Pingali P, Watson R, Zurek M (2006). Anthropogenic drivers of ecosystem change: An overview. *Ecological Society*, 11: 29.
- Oltra, R and Todolf R (1997). Effects of temperature, salinity and food level on the life history traits of the marine rotifer *Synchaeta cecilia valenrina*, n. subsp. *Journal of Plankton Research*, 19 (6): 693-702.
- Parker LM, Ross PM, O'Connor WA (2010). Comparing the effect of elevated pCO₂ and temperature on the fertilization and early development of two species of oysters, *Marine Biology*, 157: 2435–2452.
- Parker LM, Ross PM, O'Connor WA (2009). The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850), *Global Change Biology*, 15: 2123–2136
- Peck MA, Holste L (2006). Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success of *Acartia tonsa* (Calanoida: Copepoda): optimizing intensive cultures. *Aquaculture*, 255:341–350.
- Peters J (2006). Lipids in key copepod species of the Baltic Sea and North Sea—implications for life cycles, trophodynamics and food quality. PhD thesis, University Bremen, Bremen, 159 p
- Peters J, Dutz J, Hagen W (2007) Role of fatty acids on the reproductive success of copepod *Temora longicornis* in the North Sea. *Mar*
- Peters DS and Boyd MT (1972). The effect of temperature, salinity, and availability of food on the feeding and growth of the Hogchocker, *Trinectes Maculatus* (Bloch & Schneider). *Journal of experimental marine Biology and Ecology*, 7: 201-207

Perry AL, Low PJ, Ellis JR and Reynolds JD (2005). Climate Change and Distribution Shift in Marine Fishes. *Science*, 308 (5730): 1912-1915

Pisias NG, Mixa AC, Heusser L (2001). Millennial scale climate variability of the northeast Pacific Ocean and northwest North America based on radiolaria and pollen *Quaternary Science Reviews* 20: 1561–1576.

Richmond C, Marcus NH, Sedlacek C, Miller GA, Oppert C. (2006). Hypoxia and seasonal temperature: Short-term effects and long-term implications for *Acartia tonsa dana*, *Journal of Experimental Marine Biology and Ecology* 328: 177– 196.

Roemmich D, McGowan JA (1995). Climatic warming and the decline of zooplankton in the California current. *Science*, 267:1324–1326.

Saiz E, Tiselius P (1993). Experimental records of the effects of food patchiness and predation on egg production of *Acartia tonsa*. *Limnology and Oceanography*, 38(2): 280-289.

Sedlacek C, Marcus NH (2005). Egg production of the copepod *Acartia tonsa*: The influence of hypoxia and food concentration. *Journal of Experimental Marine Biology and Ecology* 318: 183–190.

Utne ACW (1997). The effect of turbidity and illumination on the reaction distance and search time of the marine planktivore *Gobiusculus flavescens*. *Journal of Fish Biology*, 50: 926–938.

Vidal J (1980). Physioecology of Zooplankton. I. Effects of Phytoplankton Concentration, Temperature, and Body Size on the Growth Rate of *Calanus pacificus* and *Pseudocalanus* sp. *Marine Biology* 56: 111-134.

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004). Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *OIKOS* 104: 451 - 457

Walsh PJ, Milligan CL (1989). Coordination of metabolism and intracellular acid-base status: ionic regulation and metabolic consequences. *Canadian Journal of Zoology*, 67: 2994–3004.

Wiltshire KH, Manly BFJ (2004). The warming trend at Helgoland Roads, North Sea: phytoplankton response. *Helgoland Marine Research*. 58 (4): 269-2