

Hydranth structure and digestion rate as a function of temperature and salinity in *Clava multicornis* (Cnidaria, Hydrozoa)

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KURZFASSUNG: Hydranthenstruktur und Verdauungsrate als Funktion von Temperatur und Salzgehalt bei *Clava multicornis* (Cnidaria, Hydrozoa). Erbgleiche Polypen von *Clava multicornis* FORSKÅL wurden in 12 verschiedenen Temperatur-Salzgehalts-Kombinationen durch asexuelle Vermehrung zu neuen Kolonien herangezogen und an den adulten Hydranthen dieser erbgleichen „Sekundärkolonien“ Länge und Breite des Hydranthenkörpers, Anzahl und Länge der Tentakel sowie die Verdauungsgeschwindigkeit ermittelt. Alle gemessenen Parameter verändern sich – zum Teil sogar erheblich – als Funktion von Temperatur und Salzgehalt. Die Veränderungen der Hydranthendimensionen führen zu Verschiebungen der Oberfläche-Volumen-Quotienten. Da der Stoffaustausch zwischen Kolonie und Umwelt primär im Bereich der Hydranthen erfolgt, kommt diesen Verschiebungen vermutlich eine Bedeutung zu im Rahmen der Stresskompensation (nicht-genetische Adaptation). Die erzielten Ergebnisse bestätigen frühere Untersuchungen von KINNE an dem naheverwandten Hydroidpolypen *Cordylophora caspia*.

INTRODUCTION

In earlier papers KINNE (1956a, b, 1958a) has reported on the effects of various levels of temperature and salinity on hydranth dimensions and rates of food intake and digestion in the colonial athecate hydroid *Cordylophora caspia* PALLAS (syn.: *C. lacustris* ALLMAN; for nomenclatural problems consult ROCH 1924, HAND & GWILLIAM 1951). If kept under constant intensities of temperature and salinity throughout the life span of individual hydranths, the brackish water living *Cordylophora* exhibits significant differences in regard to length and width of its fully grown hydranths as well as to the number and length of their tentacles. These structural responses to environmental stress were also observed in colonies obtained from a given individual “primary” colony by cutting off single hydranths and subsequently allowing these to grow into new separate “secondary” colonies via asexual reproduction (KINNE 1957a, 1958a, b). Hydranths of such genetically identical secondary colonies showed furthermore temperature and salinity dependent differences with respect to the time required to ingest prey of defined size and quality, and the duration of its digestion (KINNE 1958a).

It was the purpose of the present investigation to subject a closely related, but more marine hydroid species to comparable experimental conditions and to test its pertinent responses. The species chosen was the colonial athecate *Clava multicornis* FORSKÅL. All experiments were conducted in our Marine Station (Meeresstation) on the island Helgoland. They were executed by Mr. G.-A. PAFFENHÖFER under the direction of the first named author.

MATERIAL AND METHODS

Material

Along the German coastline the athecate hydroid *Clava multicornis* FORSKÅL of the family Clavidae occurs both in the North Sea and in the western Baltic Sea. The material used in the present study was obtained from a culture which originated from polyps collected in the "Helgoländer Felswatt" – the shallow rocky littoral surrounding of the island Helgoland. In nature, *C. multicornis* colonies usually grow on various species of macroscopic brown algae, as well as on wood and similar substrates – more rarely on bare rocks.

Methods

All experiments were started with single polyps cut off from an individual "primary" colony and consequently having the same genetical background. The stolonial basis of each newly removed polyp was fastened to the surface area of a submerged microscopic slide by means of a fine cotton sewing thread. These threads were removed only after the polyp had firmly attached itself to its new substratum. With one polyp attached to one slide, a total of 5 or 6 slides was positioned nearly vertically in a glass dish containing 500 ± 50 ml culture medium and having a surface area at water level of some 125 cm². Through asexual reproduction the polyps gave rise to "secondary" colonies serving as test material.

Basic culture medium. A sufficient supply of aged sea water of 32‰ S was sampled near Helgoland; it was filtered through a Seitz-Filter K 5 and stored at about 20°C in 60-liter glass containers kept in complete darkness.

Temperature and salinity. Annual fluctuations of water temperature near Helgoland usually range from 3° to 17°C; salinities vary from 30 to 34‰. In the laboratory the primary colony of *C. multicornis* had been kept at 16.0°C and a salinity of 32‰. Secondary colonies were tested at 12 different combinations of constant intensities of temperature and salinity: 12°, 17° and 22°C; 16‰, 24‰, 32‰ and 40‰ S. In order to avoid or reduce shock reactions or other undesired consequences of sudden environmental change, final test combinations were approached in small steps (1° or 2° C and less than 4‰ S per day). Recordings started 9 days after the initial changes of temperature and salinity. During the experiments water temperatures were maintained within $\pm 0.1^\circ\text{C}$, salinities within $\pm 1^\circ\text{‰}$. Salinity was determined using the MOHR specific gravity balance. The basic culture medium of 32‰ S

was diluted to 24 or 16‰ by adding appropriate amounts of rain water; it was concentrated to 40‰ S by adding salts (6.52 g NaCl, 1.60 g MgSO₄ × 7 H₂O, 1.24 g MgCl₂ × 6 H₂O, 0.56 g CaCl₂ × 6 H₂O, 0.23 g KCl) to 1000 ml culture medium. In order to meet the dangers of increased mortality in the extreme salinities, 10 colonies each were used in the 16 and 40‰ S combinations and 6 colonies each in 24 and 32‰ S combinations.

Light. Lux meter reading proved that all cultures were exposed to practically identical light conditions. They were kept in laboratories with large – mostly closed – glass windows; direct sunlight was avoided. Lux meter readings taken at 12 : 00 noon under conditions of a cloudless blue sky amounted to 700 at water surface of the glass dishes. The experiments were carried out from April to June, 1965; day lengths varied accordingly.

Food. *Clava multicornis* had been observed to feed on various small crustaceans, annelids, mollusk meat and small fish larvae; in fact it does not seem to be very selective. In our experiments 4-day old larvae of brine shrimp *Artemia salina* were used as food source. The brine shrimp larvae were hatched and maintained in sea water of 32‰ S at approximately 20° C and fed with unicellular algae (*Dunaliella* sp.).

Routine procedure. Each day 500 to 600 *Artemia* larvae were offered to the 5 or 6 *Clava* colonies growing on their separate slides in a glass dish. On this abundant supply the colonies were allowed to feed one hour, while the culture dishes were darkened so as to avoid or reduce aggregations of *Artemia* larvae due to phototaxis. Subsequently, the remaining brine shrimp larvae, food rests and other debris were carefully removed with pipettes and the culture medium renewed.

Measurements of hydranth dimensions were done on fully grown adult hydranth individuals – 20 hours after the last feeding – at magnifications between 12.5 and 50. Colonies were left undisturbed for at least 5 minutes before hydranth dimensions were recorded.

RESULTS

At 12° and 17° C the four salinity levels employed proved to be well within the ranges tolerated by *Clava multicornis*. At 22° C polyps survived only about 5 days in 16‰ and 7 days in 40‰ S; consequently, no data are presented for these extreme combinations.

Dimensions of hydranth bodies

The total length of fully grown hydranths (straight line distance from the hydranth's base to the tip of its mouth) varies significantly in the various temperature-salinity combinations. As can be seen from Figure 1, in 16, 24 and 32‰ S, hydranths are longest at 12° C, followed by 17° C and finally 22° C. A constant temperature of 22° C obviously represents a physiological stress even at the moderate salinity intensities of 24 and 32‰ S respectively. In regard to the four salinity levels, there is –

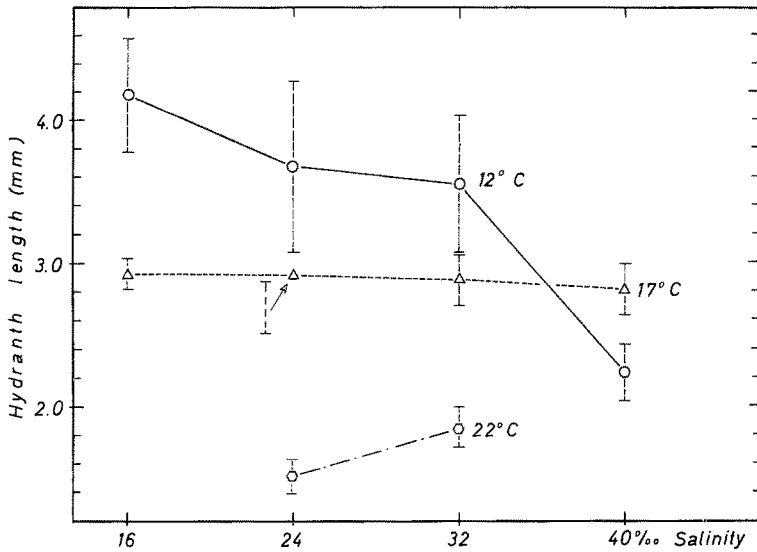


Fig. 1: Total length of adult *Clava multicornis* hydranths grown at different combinations of temperature and salinity. Each value represents the average of 10 measurements on different individuals. Dotted vertical lines: standard deviations of the means

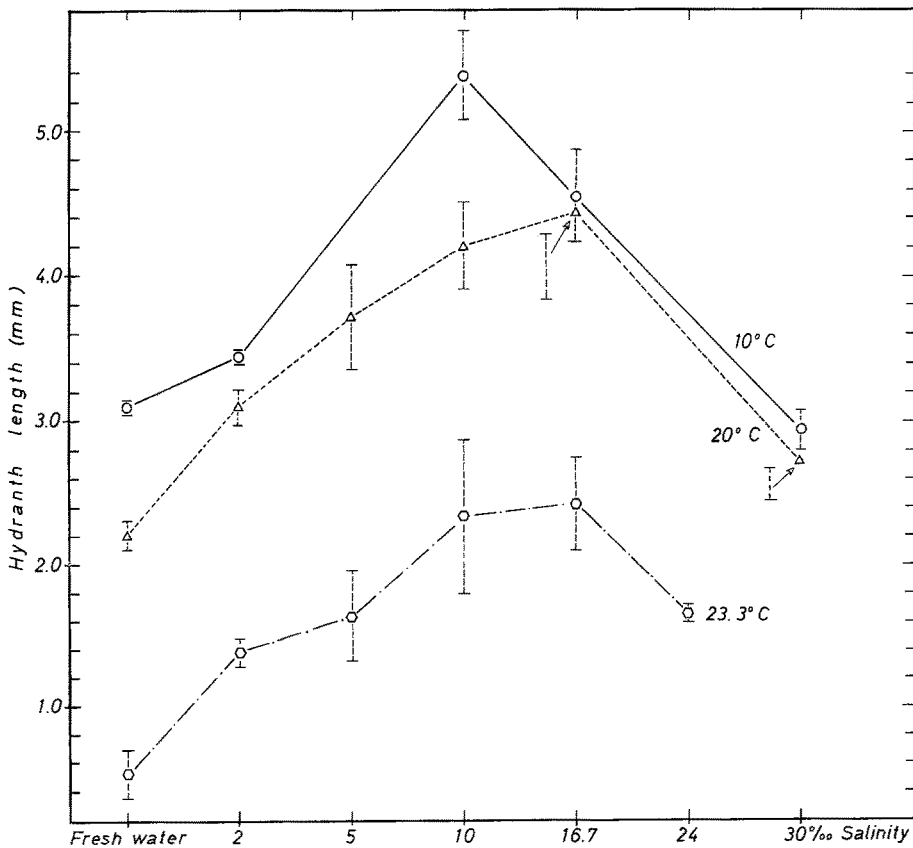


Fig. 2: Total length of adult *Cordylophora caspia* hydranths grown at different temperature-salinity combinations. Each value represents the average of 6 to 8 measurements on different individuals. With respect to standard deviations of the means, consult KINNE 1958a, footnote p. 409. (After KINNE 1956a)

with increasing salinity – a definite decline in hydranth length at 12°C, little or no decline at 17°C and an increase in length from 24 to 32‰ S at 22°C.

These results compare well with those obtained on *Cordylophora caspia* (Fig. 2). Both species respond to increasingly higher temperature levels by a progressive reduction of their hydranth length. At the same time, lower temperature levels tend to shift salinities producing maximum hydranth lengths downward while higher temperature levels tend to shift them upward. Similar positive effects of such low/low and high/high combinations of temperature and salinity have been reported also for other species (KINNE 1964, p. 324; see also discussion). The lengths of *Clava* hydranths are reduced to a lesser extent by higher salinities than those of *Cordylophora* hydranths. This was to be expected since *Clava* lives in more marine habitats than *Cordylophora*. At 12° and 17° C respectively, the lower critical salinities, which might be expected to reduce hydranth length of *Clava* again, lie apparently below 16‰.

Maximum hydranth width (greatest straight line distance across the hydranth's body) tends to decrease somewhat with increasing temperature (Fig. 3), although

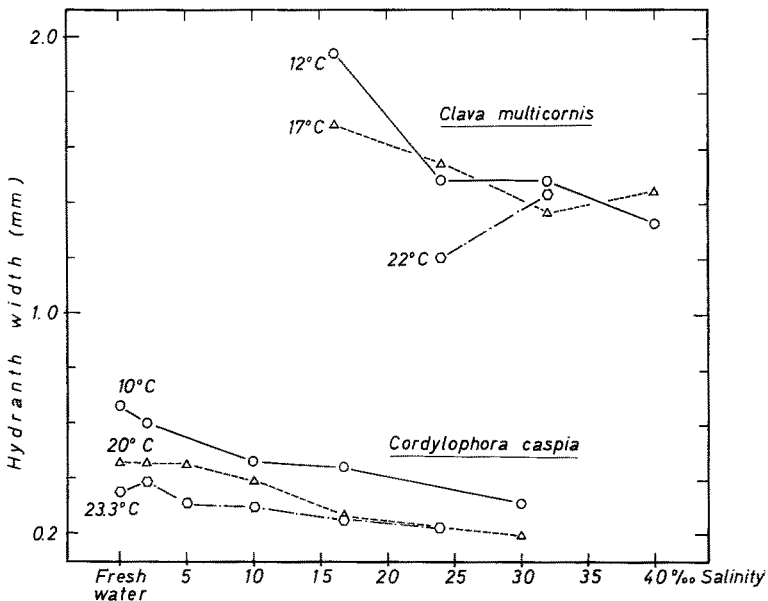


Fig. 3: Maximum width of fully grown hydranths of *Clava multicornis* (averages from 10 measurements) and *Cordylophora caspia* (averages from 6 to 8 measurements; data from KINNE 1956a). In most cases standard deviations of the means do not significantly exceed the diameters of the symbols employed to indicate mean values; they have consequently been omitted

this relationship is less obvious than the reduction of hydranth length. At 12° and 17° C hydranth width decreases with increasing salinity. This is not true, however, at 22° C, where width increases from 24 to 32‰ S.

In general, modifications in hydranth width too are quite comparable to those found in *Cordylophora* (lower part of Fig. 3), with the obvious exception concerning the marked width increase in *Clava* at 22° C from 24 to 32‰ S.

On the basis of the average values obtained for length and width, shapes of hydranth bodies of *Clava multicornis* have been reconstructed and presented diagrammatically in Figure 4 with the tentacles omitted for easier comparison. Maximum values for hydranth length and width at 12°C are obtained in 16‰, at 22°C in 32‰ S.

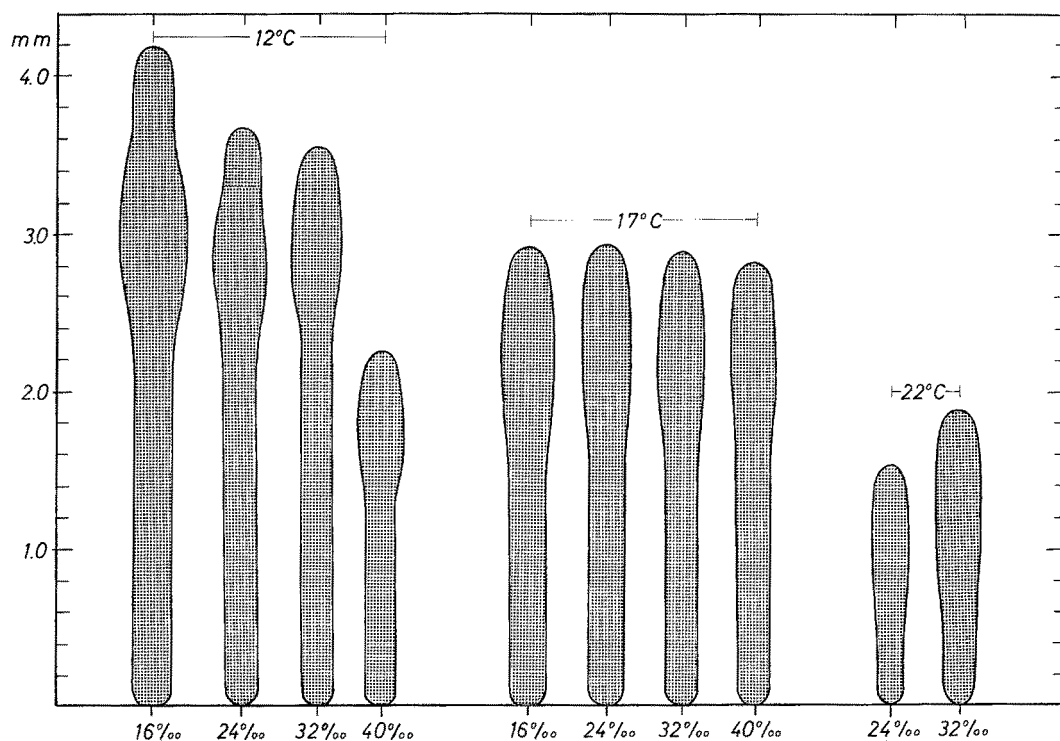


Fig. 4: Diagram showing typical shapes of *Clava* hydranths. Shapes have been reconstructed on the basis of the average values obtained for length and width

Number and length of tentacles

The number of the filiform tentacles – spaced irregularly over the surface of the hydranth body – varies considerably with temperature and salinity (Fig. 5). In general, tentacle number per hydranth tends to be positively related to the size of the hydranth body. It decreases with increasing temperature. The salinity level producing maximum tentacle numbers varies with temperature: at 12°C most tentacles are carried in 16‰, at 17°C in 24‰ and at 22°C in 32‰ S. Here again, low/low and high/high temperature-salinity combinations tend to produce maximum values: the salinity producing the highest tentacle numbers shifts upward with increasing temperature.

Tentacle length is a difficult dimension to measure. It was taken only from the tentacles of the hypostome region of undisturbed and obviously relaxed specimens.

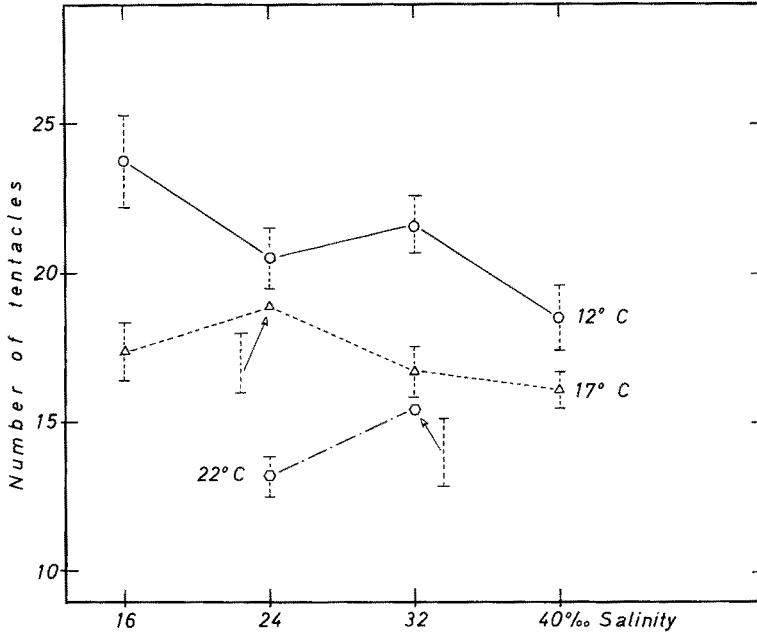


Fig. 5: Number of tentacles per adult *Clava multicornis* hydranth (averages from 10 measurements)

Figure 6 shows that tentacle length follows a similar pattern as does tentacle number. However, tentacle length attains relatively higher values at 17° C, especially in 24 ‰ S.

The corresponding values for *Cordylophora* are presented in Table 1. Here too tentacle number decreases with temperature (from 10° to 20° C); tentacle length, however, is greater at 20° C than at 10° C, except in fresh water. At both temperature

Table 1

Number and length of tentacles per fully grown hydranth in *Cordylophora caspia* as a function of temperature and salinity. Average values from 25 individual measurements. *P*: rounded off *P*-values determined graphically according to PÄTAU (1943). (After KINNE 1958a)

Water temperature	Salinity	Tentacle number	<i>P</i>	Tentacle length (mm)	<i>P</i>
10° C	fresh water	33		2.3	
	15 ‰	38	0.001	3.4	0.0003
	30 ‰	27	0.0009	1.7	0.0002
20° C	fresh water	22		2.2	
	15 ‰	31	0.0008	4.0	0.0001
	30 ‰	23	0.0009	2.3	0.0002

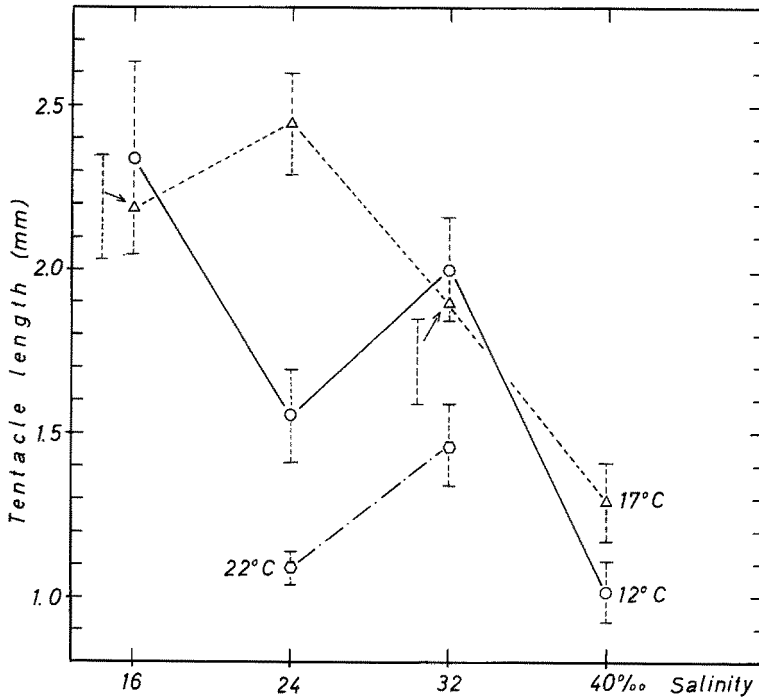


Fig. 6: Tentacle length in *Clava multicornis* (averages from 10 measurements)

levels, maximum numbers and lengths of tentacles are attained in the intermediate salinity level, namely, 15‰ S.

Time required for digestion

The time required for digestion is taken here to be identical with the time span elapsing between completed food ingestion (closure of mouth rim over the swallowed prey) and completed defecation. Obviously a detailed assessment of digestion rates would require additional and more refined techniques. The fully grown test hydranths were fed *Artemia* larvae of a length (straight line distance from anterior tip of the head to base of caudal furcae) of 1.40 mm (averages from 25 measurements performed on a group of larvae of identical age and environmental history). Previously to being fed, all hydranths had been starved for 24 hours. Completed food ingestion was determined to the nearest 5 minutes; defecation of food rests was recorded at intervals of 10 minutes.

As was to be expected, the time required for digestion decreases with increasing temperature in the order 12°, 17° and 22° C. Time required for digestion at 22° C is approximately half that required at 12° C (Fig. 7). Of special interest is the fact that – irrespective of temperature or hydranth size – the time required for digestion is shortest in the 32‰ salinity level. At both 12° and 17° C the longest digestion periods were

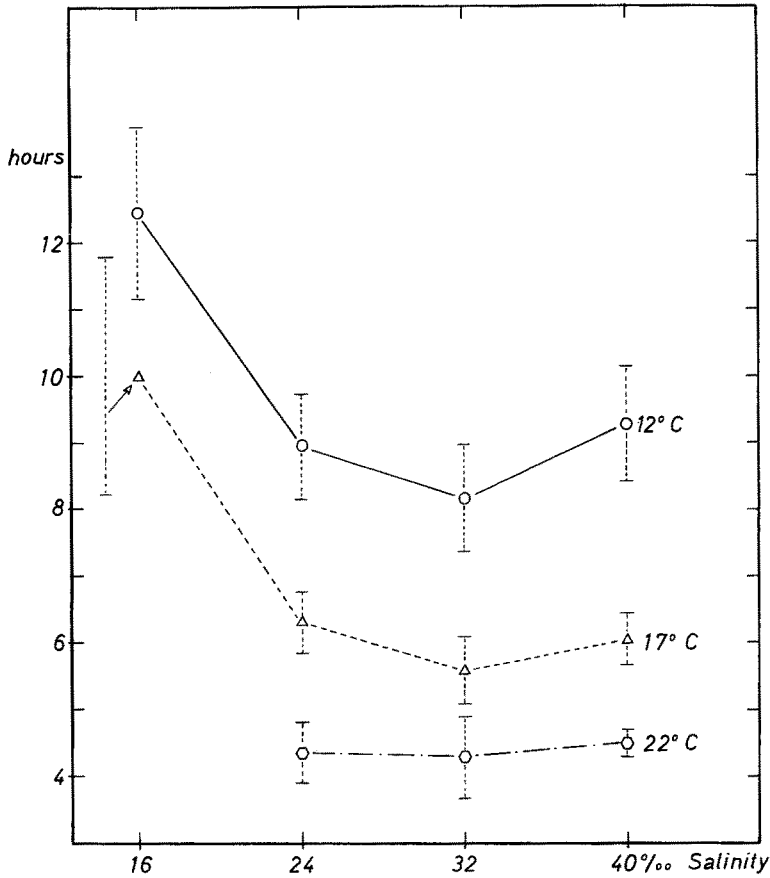


Fig. 7: Time required for digestion of a 1.40 mm long *Artemia* larva (averages from 25 measurements)

recorded in 16 ‰ S; within each temperature level, 24 and 40 ‰ S led to more or less equally long periods.

A few similar tests had been conducted on *Cordylophora* at 13°C in fresh water, 16.7 and 30 ‰ S. Here too digestion time is shortest in the intermediate salinity level of 16.7 ‰, followed by 30 ‰ and then fresh water. The period elapsing between the moment the prey is caught by the tentacles and the mouth rim is closed again behind the swallowed prey (time required for food ingestion) is affected in the same way. At 13°C average ingestion values for *Cordylophora* are: 13.0 minutes in 16 ‰, 16.1 minutes in 30 ‰ and 21.8 minutes in fresh water (KINNE 1958a, p. 426/7).

DISCUSSION

Clava multicornis lives in habitats usually characterized by fluctuations of environmental parameters – especially temperature and salinity – which are less pronounced

than those in locations inhabited by *Cordylophora caspia*. Nevertheless, *Clava* is capable of withstanding wide ranges of temperature and salinity. In the littoral region of Helgoland, the natural habitat of the *Clava* colonies tested in this study, water temperatures normally range from 3° to 17°C throughout the year, and salinities fluctuate between 30 and 34 ‰. When stepwise transferred (within a few days) into more extreme temperature-salinity combinations, representatives of the Helgoland population proved to be able to live at temperatures up to 22°C (in 24 and 32 ‰ S) and in salinities up to 45 ‰ (at temperatures between 12° and 20°C) without apparent harm for several months.

The salinity range tolerated by *Clava multicornis* from the Baltic Sea near Kiel (habitat salinity: 15 to 17 ‰) is lower. PALMHERT (1933), who transferred representatives of the Kiel population directly into the test salinities and recorded the salinity level in which his plankton-fed colonies survived for at least three weeks, found the tolerance range to be 10 to 28 ‰ S at 19° to 22°C and 9 to 32 ‰ S at 14° to 15°C. More detailed and extensive studies concerning lethal limits, critical intensities for growth and reproduction as well as for other functional and structural parameters of North Sea versus Baltic Sea colonies promise important results, especially if such comparative studies would include long term acclimation and transplantation experiments.

Cordylophora caspia obtained in 1955 from the Kiel Canal near Rendsburg (normal annual range of water temperature: about 1.5° to 20°C; salinity fluctuations between 5 and 10 ‰) may – after life-long acclimation – complete its life cycle between temperatures of 8° and 20°C (“normal temperature range”) in salinities between 1 and 24 ‰. Within its normal temperature range *Cordylophora* may tolerate fresh water and sea water of 35 ‰ for months (KINNE 1956a, b).

Prolonged existence in such relatively wide ranges of temperature and salinity results in considerable structural and functional modifications. For *Clava multicornis* the present study has demonstrated that hydranth length and width, number and length of tentacles, as well as digestion rate vary significantly in the temperature-salinity combinations employed. These modifications are similar to those previously observed in *Cordylophora*. In both species the structural variations bring about alterations in surface to volume ratios of the individual hydranths – the major sites of metabolic exchanges between colony and environment. Such alterations may conceivably affect rate and efficiencies of metabolism and represent means of compensation for environmental stress (KINNE 1957a, b, 1958a, b). The capacity for such structural non-genetic adaptation may well play an important role in mastering the thermal and osmotic stresses occurring in the habitats of both species. Changes in number and length of tentacles may possibly also affect food acquisition and the ability to overcome big and powerful prey.

With respect to hydranth body dimensions and number and length of tentacles, combinations of low temperatures and low salinities and of high temperatures and high salinities tend to yield maximum values. The same basic relationships have been found to hold for *Cordylophora* (KINNE 1956a, 1958a). Even though studies on the combined effects of temperature and salinity are still few in number, there are some other examples available concerning different phyla and different biological criteria.

Interestingly enough, in most cases studied thus far, the test organisms can tolerate subnormal salinities better at the lower part of their temperature range and often can tolerate supranormal salinities better at the upper part of their temperature range. Such beneficial effects of low/low and high/high combinations have been reported, for example, for the amphipod *Gammarus duebeni* (KINNE 1952, 1953), the crab *Rhithropanopeus harrisi* (syn.: *Heteropanope tridentatus*) (KINNE & ROTTHAUWE 1952), and the fish *Cyprinodon macularius* (KINNE 1960). Other species seem more successful in low/high or high/low combinations; for examples consult the recent review by KINNE (1964, p. 324). Theoretical considerations concerning temperature and salinity and their physiological and biological importance have led KINNE (1956c) to suggest that the density of the water may provide one key to the understanding of some aspects of such relationships.

The time required for digestion of an *Artemia* larva of a given length decreases with increasing temperature but at all temperature levels tested is shortest in 32‰ S. This fact may be related to long-term salinity adaptation. As has been pointed out, salinities near Helgoland normally fluctuate between 30 and 34‰.

SUMMARY

1. Responses to 12 different combinations of constant levels of temperature and salinity were tested in the colonial athecate hydroid *Clava multicornis*. Criteria measured were: (a) length and width of hydranth bodies, (b) number and length of tentacles and (c) rate of digestion.
2. Test colonies were obtained by allowing single hydranths – cut off from an individual “primary” colony – to regenerate via asexual reproduction into new “secondary” colonies.
3. In the resulting – genetically identical – material, all criteria tested vary as a function of the different environments offered.
4. In 16, 24 and 32‰ S hydranth length reaches maximum values at 12°C, followed by 17°C and then 22°C. With increasing salinity, hydranth length declines markedly at 12°C, while there is little or no decline at 17°C and a definite increase from 24 to 32‰ S at 22°C.
5. Hydranth width varies less extensively; in general, it follows similar patterns as does hydranth length.
6. Tentacle number per hydranth tends to be positively related to the size of the hydranth body; it decreases with increasing temperature. Salinity levels producing maximum tentacle numbers vary with temperature; maxima are found at 12°C in 16‰, at 17°C in 24‰ and at 22°C at 32‰ S.
7. Tentacle length – although a more variable criterion – is affected similarly to tentacle number; it attains, however, relatively higher values at 17°C.
8. In regard to the hydranth dimensions measured, combinations of low temperatures/low salinities and of high temperatures/high salinities tend to produce maximum values.
9. Rate of digestion is taken here to be identical to the time elapsing between com-

- pleted food intake and defecation. This time span is reduced with increasing temperature. In all temperature levels, digestion time is shortest in 32‰ S.
10. The results presented above compare well with those obtained earlier under similar conditions on *Cordylophora caspia*.
 11. It is assumed that the structural modifications of hydranths affect rate and efficiency of exchanges between organism and environment and may thus represent a means of metabolic adjustment.

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