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Instantaneous salinity reductions affect the survival and feeding rates of the co-occurring copepods *Acartia tonsa* Dana and *A. clausi* Giesbrecht differently

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ABSTRACT

Salinity variability at short time scales constitutes a severe restriction to marine life in coastal and estuarine ecosystems. In these environments zooplankters may experience rapid salinity variations due to diverse processes, yet lethal or sub-lethal responses to such changes have been scarcely studied. We assessed short-term (12 h) survival and time-integrated clearance (F; mL ind^{-1} h^{-1}) and ingestion rates (I, μgC ind^{-1} h^{-1}) after 1, 2, 4, 8 and 12 h of two widespread and abundant coastal copepods, *Acartia tonsa* and *A. clausi*, subjected to instant salinity changes from 32 PSU to 26, 20, 14, 8 and 4 PSU (*A. tonsa*) and from 32 to 26, 20 and 14 PSU (*A. clausi*). We expected that *A. tonsa*, which occur naturally in environments where sharp salinity gradients are common would tolerate wider salinity changes than *A. clausi*, which less frequently encounter sharp gradients in nature. For *A. tonsa* mortality for the extreme haline shock (change from 32 to 4 PSU) was 31%, whereas *A. clausi* reached 22% mortality already at a change from 32 to 14 PSU; in comparison, mortality for *A. tonsa* at the 32/14 PSU treatment was only 3%. F and I decreased significantly at extreme treatments, and the total clearance in experimental bottles with salinity shocked animals (F_{tot}, mL h^{-1}) was only 5% of rates measured in non-shocked control bottles for *A. tonsa* (32/4 PSU change) and 20% for *A. clausi* (32/14 PSU change); corresponding total ingestion (I_{tot}, μgC h^{-1}) represented 9.5% of that in control bottles for *A. tonsa* and 24% for *A. clausi*. In comparison, the 32/14 PSU treatment did not affect either clearance or ingestion rates in *A. tonsa*. Results suggest that in the field *A. tonsa* is not likely to suffer significant mortalities due to sudden salinity reductions in the surrounding medium — except under extreme circumstances— while *A. clausi* cannot tolerate changes >18 PSU. However, in both species feeding activity could be severely compromised by salinity reductions. The decreased feeding rate may have direct implications for processes ranging from energy acquisition at individual level to organic matter transfers at ecosystem level and thus deserves more attention in experimental studies and population modelling.

1. Introduction

Estuaries and coastal waters constitute highly productive systems where diluted seawater imposes severe physiological restrictions to marine plants and animals (Kinne, 1971; Soetaert and Herman, 1994; Attrill, 2002; Nielsen and Andersen, 2002). Planktonic copepods are the major trophic link between primary producers and large-sized consumers in pelagic food webs. Amongst the few copepod taxa that tolerate significantly diluted seawater are a number of calanoid species belonging to the genus *Acartia* present in estuaries and coastal waters globally.

Salinity variability at short time-scales, for instance during a tidal cycle, may constitute a stressor equally or even more important than constantly low levels, and may explain the distribution of estuarine fauna better than mean salinity values (Remane, 1934 c.f. Attrill, 2002; Remane and Schlieper, 1971). Frequent shifts in salinity may impose additional physiological stress leading to acute mortality, impaired population growth and shifts in the functioning of food webs (Kaartvedt and Aksnes, 1992; Soetaert and Herman, 1994; Attrill, 2002). For zooplankters, short-time salinity variations may occur due to rapid dilution of surface waters for instance during heavy precipitation or ice/snow meltoff in the catchment area (e.g., Ambler, 1985), due to strong mixing caused by storms, or as a result of their own vertical migration in a stratified water column. Copepod carcasses can constitute nearly one third of total copepod abundance (Weikert, 1977; Tang et al., 2006), and significant non-predatory mortality as a result of rapid salinity changes in the field have been observed (Kaartvedt and Aksnes, 1992; Soetaert and Herman, 1994, Hubareva et al., 2008). Copepods may also become entrained in the compensation current of the two-layer baroclinic circulation typical of

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stratified estuaries/fjords and be transported towards the head of the fjord. Here, salinity differences between distinct vertical layers can be up to 30 PSU over a few meters (i.e., Uncles and Stephens, 1996; Vieira and Bordalo, 2000; Andersen and Nielsen, 2002; Nielsen and Andersen, 2002). The rate of salinity change actually experienced by a zooplankter will depend on the mechanism involved and may be expected to range from an almost instantaneous shock to a change smoothed over tens of seconds, a few minutes or hours. One extreme circumstance is when a ship releases its ballast water containing planktonic organisms, and the ability to endure osmotic shocks may be one trait affecting the probability that species carried in the ballast water survive in the new environment.

With the exception of the estuarine copepod *Eurytemora affinis* (e.g., Von Vaupel-Klein and Weber, 1975; Lee and Petersen 2002, 2003; Lee et al., 2003), studies of lethal effects of sudden salinity changes on estuarine and coastal pelagic copepods are scarce (Lance, 1963a,b; Bhattacharya, 1986; Ough and Bayly, 1989; Cervetto et al., 1999; Chen et al., 2006). There are very few published quantitative data from controlled experiments considering both lethal and sub-lethal responses to acute salinity changes (e.g., respiration and excretion, Farmer and Reeve, 1978). Feeding constitutes the basic process providing energy for all vital functions and is a core mechanism of organic matter fluxes in ecosystems. Knowledge of factors regulating feeding of organisms is thus central for understanding processes from individual to system levels. Modulation of copepod feeding rates by salinity has been addressed by a few experimental studies solely on *Acartia* spp. (Gaudy et al., 2000; Calliari et al., 2006) and *E. affinis* (Powell and Berry, 1990). However, salinity shock effects on feeding were assessed only in the study by Lance (1964) and only semi-quantitatively. Results indicated diminished feeding (estimated as faecal pellet production) in *A. discaudata* and *A. bifilosa* beyond a threshold of 50% seawater dilution.

Information on the responses of abundant copepod species to rapid salinity changes (i.e., without pre-acclimation) can complement current knowledge on zooplankton response to salinity and contribute to a better understanding of how this important variable affects the structure and functioning of estuarine and coastal planktonic ecosystems. *Acartia tonsa* and *A. clausi* are widespread species in estuaries of Europe and America and both species have been subject to a large number of laboratory studies (see Mauchline, 1998 for references), including some on tolerance and responses to salinity. In the field, *A. tonsa* can be found from <5 up to ca. 30 PSU (Cervetto et al., 1999, and references therein), and can tolerate a wide salinity range from nearly 0 (Cronin et al., 1962) to 52 PSU (Rey et al., 1991); *A. clausi* is usually restricted to areas under direct marine influence and has narrower salinity tolerance, with a minimum close to 15 PSU (Castro-Longoria, 2003; Calliari et al., 2006). Studies with salinity acclimatised animals showed that wider salinity tolerance in *A. tonsa* is linked to a more stable energetic balance at low salinities (i.e., gross growth efficiency, cost of growth), and better performance in terms of feeding, egg production rate, egg hatching success and naupliar survival over a wider salinity range (Lance, 1963a; Lance, 1964; Gaudy et al., 2000; Castro-Longoria, 2003; Chinnery and Williams, 2004; Calliari et al., 2006).

We performed laboratory experiments to assess and compare survival and feeding responses of *Acartia tonsa* and *A. clausi* subjected to instant salinity changes. Our hypothesis was that *Acartia tonsa* which occurs naturally in areas where sharp salinity gradients are frequent, for instance, in middle reaches of estuaries, will be able to survive wider instant salinity changes and will have a more stable feeding response than *Acartia clausi* – a species occurring preferentially at higher salinities and encountering sharp gradients less frequently.

2. Materials and methods

We exposed adult females of *Acartia tonsa* and *Acartia clausi* to various degrees of instantaneous salinity decrease, i.e., salinity shock, and measured mortality and feeding rates (as clearance, F, mL ind^{-1} h^{-1}; and ingestion, I, μgC ind^{-1} h^{-1}). Salinity was controlled using an electronic sensor (WTW LF 196 salinometer) and values are reported as practical salinity units (PSU).

2.1. Experimental animals

Acartia tonsa were obtained from a laboratory culture originating from Øresund, Denmark and kept for several generations at 32 PSU (±0.5 PSU, below-pycnocline water, BPW, from Gullmarsfjorden, Sweden). *Acartia clausi* were obtained from a culture of animals from Gullmarsfjorden collected one month prior to the experiments. The culture was kept in BPW adjusted to 23 PSU (*in situ* surface salinity at the time of collection). The copepod cultures were kept at 19 °C (±1 °C) and fed a mixture of *Thalassiosira weissflogii* and *Rhodomonas baltica* provided *ad libitum*. Before the experiments, recently moulted adult *Acartia clausi* females were acclimatised to 32 PSU in 3 steps of 3 PSU with 48 h between the steps and the last step (from 29 to 32 PSU) conducted 48 h before the experiments. One day prior to the experiments individuals were picked from the cultures and acclimatised to experimental food conditions.

2.2. Experiments

Water dilutions were prepared by mixing appropriate volumes of 0.45 μm filtered BPW and distilled water, and final salinity was checked using the salinometer. Temperature during the experiments was 19 °C (±1 °C, similar to the cultures).

During the experiments copepods were fed the diatom *Thalassiosira weissflogii* Fryxell & Hasle (strain CCAP 1085/1 reared at Göteborg

Table 1
Acartia tonsa and *A. clausi:* Setup for salinity shock experiments including shock strength, number of individuals, duration, incubation volume and initial food concentration used

Species	Shock treatment (PSU)	Vol (mL)	# fem	Duration	Initial food concentration (μgC L^{-1})		Average food concentration (μgC L^{-1})		Sampling for cell concentration	Mortality shock / control
					control	shock	control	shock	time (h)	%
A. tonsa	32 to 26	100	8	12 h	135	143	102	114	1 –2 – 4 – 8 – 12	0 / 6
A. tonsa	32 to 20	60	12	4 h	178	176	122	143	1 – 2 – 4	0 / 2
A.tonsa	32 to 20	100	8	12 h	169	159	125	137	2 – 4 – 8 – 12	6 / 3
A. tonsa	32 to 14	100	8	12 h	149	151	121	163	1 –2 – 4 – 8 – 12	3 / 3
A. tonsa	32 to 8	100	8	12 h	159	156	112	135	1 –2 – 4 – 8 – 12	3 / 0
A. tonsa	32 to 4	100	8	12 h	161	167	99	175	1 –2 – 4 – 8 – 12	31 / 3
A. clausi	32 to 26	100	8	12 h	165	164	137	145	1 –2 – 4 – 8 – 12	0 / 0
A. clausi	32 to 20	100	8	12 h	156	188	129	188	1 –2 – 4 – 8 – 12	0 / 0
A. clausi	32 to 14	100	8	12 h	166	174	150	174	1 –2 – 4 – 8 – 12	0 / 22

Average food concentrations are geometric means of start and final values, and in all cases correspond to average estimates of 4 replicate bottles.

University Marine Algal Culture Centre, GUMACC) from batch cultures at exponential growth and supplied at 150 µg C l^{-1}. That is below food saturation level for both *Acartia* species (Berggreen et al., 1988; Besiktepe and Dam, 2002). Food levels mostly decreased moderately but in two occasions increased during experiments (Table 1). Algal carbon content was estimated from cell volume according to Mullin et al. (1966), and measurements of Equivalent Spherical Diameter (average ESD, between 11.1 and 13.7 µm) using an electronic particle analyzer (Elzone 5380, 95-µm orifice tube). Batch cultures of *T. weissflogii* were kept at salinities of 32, 20, 16 and 14 PSU (algae originally kept in BPW were acclimatised in several steps of 2 PSU down to 14 PSU). The batch kept at high salinity was used as food in controls, while the batches kept at lower salinities were used as food in the respective treatments.

For each species and treatment, a 12 h experiment was conducted (see Table 1 for experimental setup). In one case (*Acartia tonsa,* change from 32 to 20 PSU), two experiments were conducted of 4 and 12 h duration, respectively (Table 1).

As a standard procedure, before transfer to experimental bottles copepods were kept for ca. 2 h in 200 mL beakers without food. Animals were transferred during daytime instantaneously from 32 PSU to each of 4 replicate glass bottles filled with the experimental solution. For "non-shocked" controls (4 replicates), copepods were transferred to a set of bottles containing the same food suspension but at the same salinity of 32 PSU. Similarly, 2 sets of control bottles without copepods containing corresponding suspensions of algae at 32 PSU and at the "shock treatment" salinity (also 4 replicates each) were prepared. The bottles were incubated standing in the dark and after 1, 2, 4, 8 and 12 h a sample of 5 mL was taken after stirring gently with a plastic plunger. A nitex screen with a mesh-size of 90 µm was used to prevent animals from being sucked into the pipette. The sample was diluted to 10 mL with 0.45 µm filtered water of the same salinity, and the concentration of *T. weissflogii* was determined with the electronic particle analyzer. Clearance rate, F, was calculated as a time-integrated F response from the beginning of the experiment until the corresponding sampling time (1, 2, 4, 8 or 12 h) according to Frost (1972). Responses in ingestion rate, I, were calculated by multiplying F by the average food concentration during the corresponding time period. At the end of the experiments, the contents of the bottles were collected on a 50 µm sieve and the animals were recovered to qualitatively assess their condition (i.e., swimming behaviour and apparent normal escape responses) and to estimate mortality. Clearance and ingestion rates were then corrected for mortality and are thus given as rates per surviving animal.

2.3. Data analysis

Observed mortalities and estimated individual ingestion rates were analysed for each species separately by two-way ANOVA to evaluate the effects of the salinity shock ("shock" factor with two levels: comparison between shocked and respective non shocked copepod control incubated simultaneously) and the effect of the shock strength ("shock strength" factor, a comparison among shock intensities, with five levels for *Acartia tonsa* and three levels for *A. clausi*). Time-integrated F and I for the different observation times in control and treatment bottles provided a general picture of the response pattern to salinity shocks, and statistical comparison of feeding responses to salinity shocks were based on estimations of I over the 12 h period. To evaluate the potential effects of salinity shock on grazing at the population level total clearance rate (F_{tot}, mL h^{-1}) and total ingestion rate (I_{tot}, µgC h^{-1}) within each bottle were estimated as the product of individual rates times the number of surviving animals. To test the effect of shock strength on total ingestion (I_{tot}), ratios between I_{tot} in shock and control treatments (shock : control ratio) were compared for each species by ANOVA with "shock strength" as factor. Finally, the same ratios were compared between species by two-way ANOVA ("shock strength" and "species" as factors) considering only salinity treatments common for the two species (32/26, 32/20 and 32/14 PSU). Post hoc Fisher's least significant difference (LSD) test was used with ANOVA, and in all cases significance was considered when $p < 0.05$. Prior to the analyses response variables were $\log_{10}$ (I) or square root transformed (I_{tot}), and homoscedasticity checked by Hartley-Cochran-Bartlett test.

3. Results

For *Acartia tonsa,* mortality during the experiments was low: 0–6.3% in controls and 0 – 3.1% in experimental treatments, except for the 32/4 PSU treatment where 31.3% of the animals died and most of the survivors looked severely affected with slower than normal movements and/or abnormal swimming. Significant differences in mortality existed for "shock strength" and for the interaction between "shock" and "shock strength" factors, with highest mortality of animals in the extreme treatment decrease (Table 2). The pattern of mortality for *A. clausi* was similar, with no mortality in controls or in the experimental treatments except for the strongest shock treatment (32/14 PSU, 22% mortality).

With a few exceptions, time-integrated clearance and ingestion rates showed a similar pattern in both control and experimental bottles, for both species and for most shock levels (except extreme shock treatments): higher feeding during the first 2 to 4 h relative to rates integrated over longer periods (Figs. 1 and 2). Also, rates in controls were higher than (or similar to) rates in the salinity shock treatments; the differences tended to decrease with increase of the observation period length, although this was not the case for the extreme shock treatments in both species. For *A. tonsa,* the total clearance rates of experimental populations exposed to 32/8 and 32/4 PSU shock treatments were 38% and 5% of control populations, respectively (Fig. 3). For *A. clausi,* the figures were 60 and 20% for 32/20 and 32/14 treatments, respectively. Ingestion rate over 12 h showed significant effects of both "shock" and "shock strength" for *Acartia tonsa* (ANOVA, $F_{1, 30} = 105$, and $F_{4, 30} = 4.4$, respectively, $p < 0.01$ in both cases), and for *A. clausi* (ANOVA, $F_{1, 18} = 96$, $p < 0.01$, and $F_{2, 18} = 5.3$, $p < 0.05$, respectively). Furthermore, the I_{tot} shock:control ratio differed among treatments for both *A. tonsa* (ANOVA, $F_{4, 15} = 155$, $p << 0.01$) and *A. clausi* (ANOVA, $F_{2, 9} = 102$, $p << 0.01$) (Fig. 3). A comparison of the I_{tot} shock:control ratio between species showed a lower decrease in ingestion rates for *A. tonsa* than for *A. clausi* within each treatment (two-way ANOVA, salinity decrease effect: $F_{2, 18} = 16$, $p < 0.01$; species effect: $F_{1, 18} = 10$, $p < 0.01$; interaction: $F_{2, 18} = 19$, $p < 0.01$, Fig. 3). Total carbon ingested by *A. tonsa* in treatment bottles represented 46% (32/8 PSU treatment) and 9.5% (32/4 PSU treatment) of that consumed in controls; for *A. clausi* ingestion in treatment bottles represented 88 and 24% of consumption in control bottles (for 32/20 PSU and 32/14 PSU treatments, respectively).

Table 2
Acartia tonsa and *A. clausi*: Results of ANOVA performed to evaluate "shock" and "shock level" effects on mortality rates in salinity shock experiments

	Effect	S Squares	Df	M Squares	F	p
Acartia tonsa	Intercept	11.03	1	11.023	21.69	>>0.01
	Change	7.10	4	1.78	3.49	0.019
	Shock/control	1.23	1	1.23	2.41	0.13
	Change*S/C	12.40	4	3.10	6.09	0.001
	Error	15.25	30	0.51		
Acartia clausi	Intercept	2.042	1	2.04	13.36	0.0018
	Change	4.08	2	2.04	13.36	0.0003
	Shock/control	2.04	1	2.04	13.36	0.0018
	Change*S/C	4.08	2	2.04	13.36	0.0003
	Error	2.75	18	0.15		

4. Discussion

4.1. Survival after salinity shock

The survival of *A. tonsa* and *A. clausi* clearly showed that both species can withstand a significant osmotic change, although *A. tonsa* tolerated a salinity decrease of at least 10 PSU larger than *A. clausi*. These results are consistent with the observed patterns in the distribution of these two species. Better tolerance of *A. tonsa* to instantaneous osmotic changes fits well with the tendency of this species to dominate and develop maximum population abundance in low and middle salinity regions of estuaries. Here, the steepest salinity gradients generally occur due to the two-layered circulation during haline stratification (Mann and Lazier, 1996; Oliveira et al., 2006). In turn, *A. clausi* is usually found at higher salinities along the estuary and in coastal areas (Jeffries, 1962; Castro-Longoria, 2003; Lawrence et al., 2004).

Our results on the survival of both species are in good accordance with earlier findings by Lance (1963a,b), who reported that instant salinity decreases of up to 25 PSU did not kill *A. tonsa* (after 20 h) while

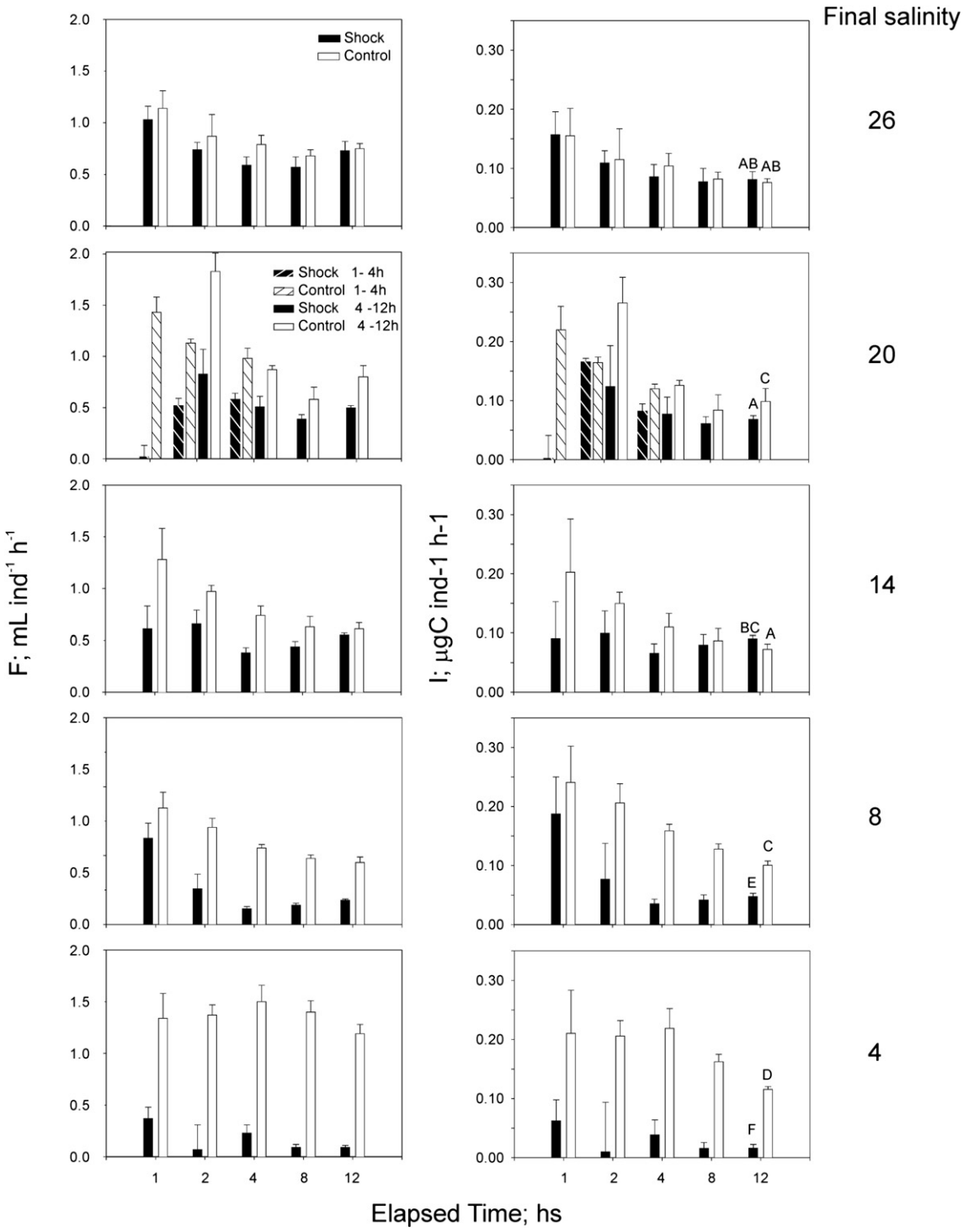

Fig. 1. *Acartia tonsa*: Individual clearance (F, mL ind.$^{-1}$ h^{-1}, left panel) and ingestion rates (I, µgC ind^{-1} h^{-1}; right panel) integrated over 1, 2, 4, 8, and 12 hrs of copepods exposed to salinity change and of controls. Bars represent means of 4 replicates; error bars represent standard deviations. Letters A–F indicate homogeneous groups (Fisher's LSD post-hoc test).

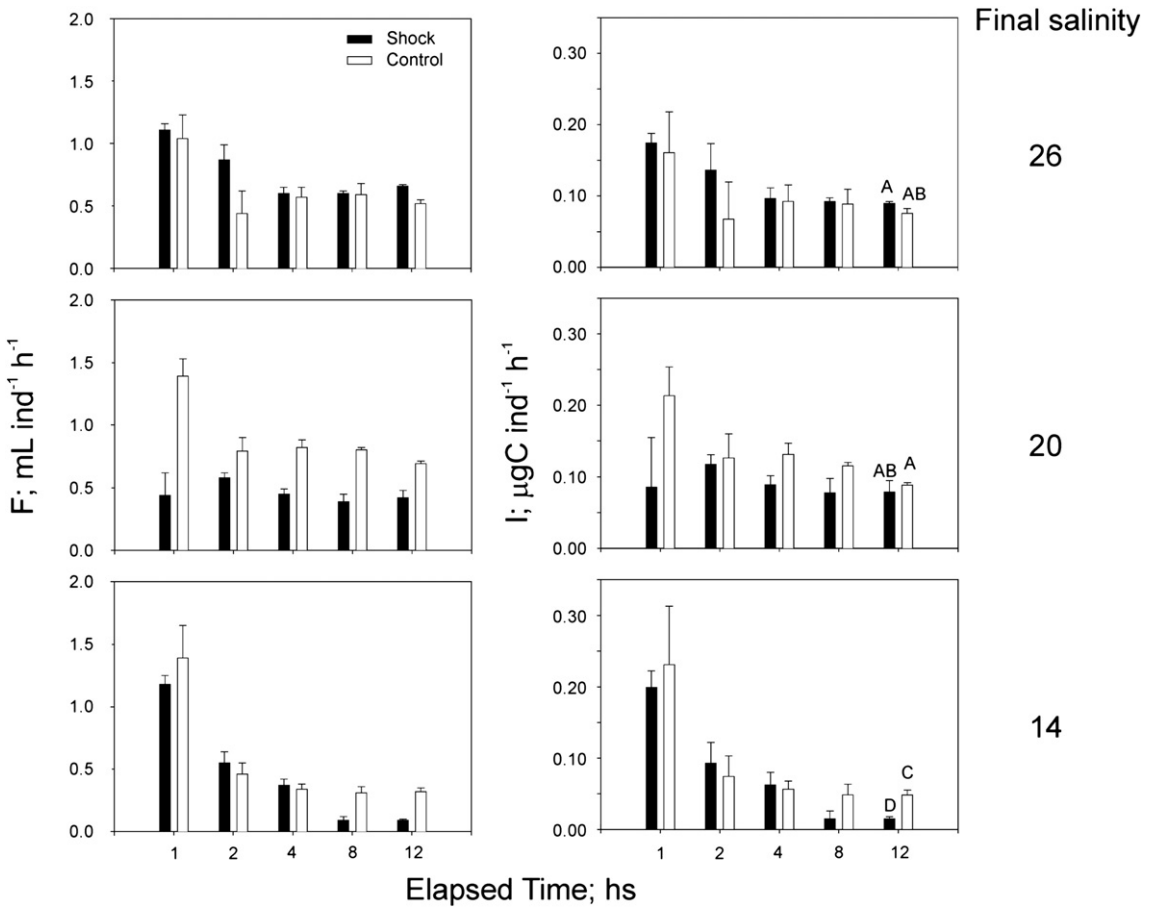

Fig. 2. *Acartia clausi*: Individual clearance (F, mL ind.$^{-1}$ h^{-1}, left panel) and ingestion rates (I, µgC ind^{-1} h^{-1}; right panel) integrated over 1, 2, 4, 8, and 12 hrs of copepods exposed to salinity change and of controls. Bars represent means of 4 replicates; error bars represent standard deviations. Letters A–D indicate homogeneous groups (Fisher's LSD post-hoc test).

decreases ≥27 PSU induced significant mortality and a decrease of 32 PSU caused death of >50 % of all experimental animals within 20 h. Interestingly, Lance observed that increased mortality rates may continue up to 8 days after the salinity change (Lance, 1963a). Moreover, these experiments showed that mortality rate increases substantially when the salinity shock is accompanied by a concurrent change in temperature (Lance, 1963a), a scenario not unlikely under natural conditions. For *A. clausi* the same author (Lance, 1963b) reported increased mortality in females for salinity decreases ≥20 PSU, and 50% mortality after 20 h was recorded at shock steps ≥23 PSU, which is consistent with the present results (25% mortality rates 12 h after a 18 PSU decrease).

Osmotic regulation capabilities are very limited in both *Acartia* species. *Acartia clausi* seems to osmoconform in the range of 24 to 32 PSU (Bayly, 1972), while *A. tonsa* is a very weak hyper-regulator (according to Lance, 1965) or an osmo-conformer (Farmer and Reeve, 1978), when exposed to diluted seawater. When exposed to diluted seawater, these copepods should counteract the problem of hydration and diffusive loss of ions at cellular level, and in such cases tolerance to salinity changes is likely determined by the capacity of cells to adjust their volume and ionic content (i.e., anisosmotic regulation of cellular fluids, Péqueux, 1995). In other crustaceans, volume control starts with a fast response phase of "swelling limitation" and proceeds with a slower "volume adjusting" phase, both of which imply modulation of internal osmolytes to bring intra- and extra-cellular fluids to an osmotic equilibrium (Péqueux, 1995). These substances include potassium, ninhydrin positive substances and the intracellular pool of free amino acids (FAA; Farmer and Reeve, 1978; Gonzalez and Bradley, 1994; Péqueux, 1995; Tirad et al., 1997). A decrease in the amino acid concentration could be achieved via catabolic activity and enhanced amino acid excretion (Farmer and Reeve, 1978), or by protein synthesis (Pierce, 1982), including salinity stress proteins as shown in a variety of organisms (Tirad et al., 1997), including copepods (Gonzalez and Bradley, 1994). Protein synthesis is energetically costly (Thor, 2000) and a previous study has shown a significant increase in respiration due to salinity stress in *A. clausi* (Calliari et al., 2006). Stress proteins may regulate solute efflux and influx processes or serve as chaperones to protect vital proteins from denaturing (Gonzalez and Bradley, 1994; Sinha and Häder, 1996). The trigger and control of such homeostatic mechanisms is poorly known but may involve internal cell osmolarity acting on key enzymes or transporters, and membrane or intracellular receptors sensing cell volume changes (Péqueux, 1995). Fast volume control should confer increased capability to survive under changing salinity regimes (Péqueux, 1995), and differences in the efficiency of these regulatory mechanisms may ultimately explain observed differences between these two copepods in their ability to cope with salinity changes.

A comparison of present and earlier results tends to support the suggestion that abrupt salinity decreases (like in the present study) are less lethal to copepods than abrupt increases (Cervetto et al., 1999). In experiments, where *A. tonsa* females were transferred from low (6–19 PSU) to a range of higher salinities (16–41 PSU) on different occasions throughout an annual cycle, significant mortality occurred for 10–15 PSU or greater steps (Cervetto et al., 1999); that is a notably smaller change that those inducing significant mortality in our (28 PSU) and other studies (Lance 1963a,b). The differential response is most probably related to the osmotic regulation mechanisms, i.e. the up- or down-regulation of the intracellular FAA pool in these osmo-

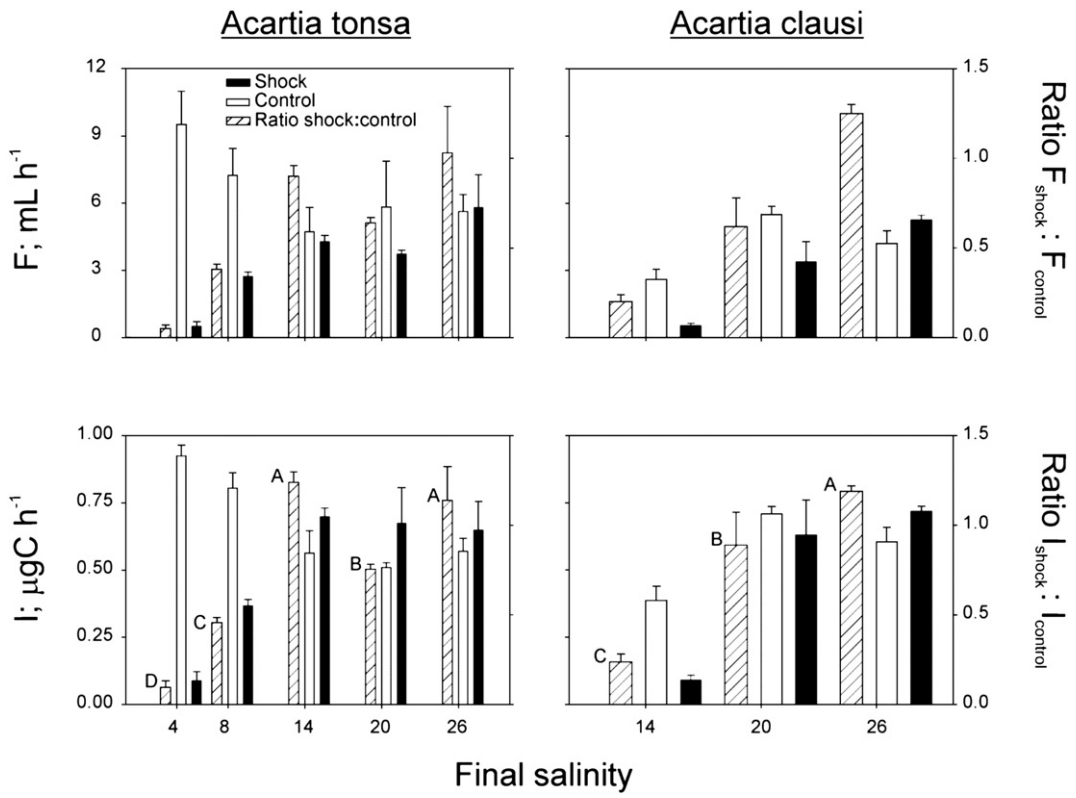

Fig. 3. *Acartia tonsa* and *A. clausi*: Total clearance (F_{tot}, mL h^{-1}; mean ± SD, left Y-axis) and ingestion rates (I_{tot}, µgC h-1; mean ± SD, left Y-axis) in experimental and control bottles and the shock to control ratio (right Y-axis) for different levels of salinity change. Letters A–D indicate homogeneous groups (Fisher's LSD post-hoc test).

conforming copepods (Farmer and Reeve, 1978). Shrinking of the FAA pool contributes to adjust internal osmolarity to decreased external salinity; in that case both protein synthesis and enhanced excretion combine to drop the FAA pool, a mechanism that works even under fasting conditions (Farmer and Reeve, 1978). On the contrary, the build up of the FAA pool to adjust to higher salinities is more dependent on amino acid intake (Farmer and Reeve, 1978), and this mechanism could be compromised if ambient food concentrations are low or if feeding itself is negatively affected by the salinity change. However, the FAA pool might also be increased by protein catabolism, which would make the process independent of feeding.

4.2. Feeding response after salinity shock

Sublethal effects on clearance and ingestion rates from the present study tended to match qualitative findings by Lance (1964), who found diminished feeding by *Acartia discaudata* and *A. bifilosa* beyond 50% seawater dilution. For both species in most treatment conditions feeding was higher during the first 2 to 4 h of incubation, while F and I responses integrated over >4 h tended to be lower and more stable. We believe this pattern may result from the 2 h pre-experimental starving period: when starved for a short period, small copepods like *A. clausi* and *A. tonsa* exhibit increased feeding activity when food is again available (Tiselius, 1998). Interestingly, even strongly stressed animals exhibited the post-starvation enhanced feeding reaction. Qualitative observations during pilot experiments showed that the immediate response of individuals to strong saline shocks (i.e., changes >12 PSU) generally involved a very short phase of violent movements of limbs and strong jumps, followed by abnormal swimming behaviour with diminished movements of all appendages and in extreme cases almost motionless sinking. That reaction typically lasted for 1 to 5 min; thereafter some animals would slowly start recovering. Our first measurements 1 h after the salinity change revealed enhanced feeding similar to that in controls except in the most extreme salinity shocks, which suggests an impressive short-term recovery capacity of these organisms.

A relevant question here is whether surviving animals, which seem to recover and resume a normal short-term feeding pattern, will survive on a longer term. Lance (1963a) showed that mortality due to salinity stress can be delayed for several days; thus it is possible that food intake continues for a short time in severely shocked animals in spite of irreversible tissue damages causing delayed mortality. Moreover, copepods with compromised swimming ability during the initial shock phase will be very vulnerable to predators in the field, such as planktivorous fish or predatory zooplankton. Indeed, zooplanktivores appear to gather in the mixing zones where the zooplankton is killed or shocked (Kaartvedt and Aksnes, 1992).

The shock-induced decreases in F_{tot} and I_{tot} reported here are indicative of potential effects on the total grazing pressure exerted by a copepod population, or a fraction of a population, subjected to a sudden salinity change. Such feeding depression may have important implications on copepod growth, reproduction and on their role and importance in the organic carbon cycling and energy transfer to higher trophic levels.

4.3. Implications for the inter-specific competition and species distribution

Haline stratification can affect vertical migration of planktonic organisms. For instance, Lance 1962 demonstrated elegantly that the fraction of a population moving across a salinity interface decreases as the salinity difference between the layers increases (Lance 1962): for *Acartia tonsa* 10% of individuals migrated from full strength sea water to a 14.4 PSU layer, while only 3% moved to a 3.6 PSU layer and none moved from full strength to a 1.8 PSU layer. For A. clausi, 9% of the experimental animals migrated from full-strength seawater to an 18 PSU layer but none to a 14.4 PSU layer.

Exposure of planktonic organisms to sudden changes in salinity can occur during mixing events (forced by tidal currents or storms or

during heavy rainfall that may dilute surface waters), or be driven by behaviour, i.e. vertical migration in a salinity-stratified water columns. Zooplankton are probably able to sense and respond to haline discontinuities in a way that attenuates osmotic shock (i.e., stay above, below, or within a halocline; Lance, 1962; Harder, 1968; Lougee et al., 2002), however they may also perform migrations across pycnocline and thereby subject themselves to salinity changes and possible osmotic stress (e.g., Cervetto et al., 1995, Morgan et al., 1997). A seaward net surface flow and a compensating deeper flow directed landward is a typical situation of stratified estuaries (Mann and Lazier, 1996) where vertical migration between the layers constitutes a mechanism enhancing retention within the system (Hough and Naylor, 1992; Morgan et al., 1997; Oliveira et al., 2006). Also, the natural food of copepods (planktonic algae, detritus and protozoans) is often patchily distributed vertically and horizontally on small scales (Jaffe et al., 1998). Copepods may localize and exploit patches of high food concentration (Tiselius 1992), which is a major asset because food is often limiting during summer (e.g., Kiørboe and Nielsen, 1994). The distribution of food is determined by the vertical physical/chemical structure of the water column and highest concentrations are often found within or above the pycnocline/halocline (Richardson and Christoffersen, 1991). This means that copepods may be forced to swim through a strong salinity (and temperature) gradient in order to optimize their feeding rates, implying a trade-off between a certain degree of osmotic stress and improved food availability. Regardless, by residing in or near the pycnocline, copepods may run greater risks of being exposed to sudden salinity changes.

It seems unlikely that *Acartia tonsa* would experience significant mortality due to salinity shock under most circumstances in the field (i.e., mixing or vertical migrations that may subject animals to salinity changes up to ca. 20 PSU). In contrast, *A. clausi* could be expected to face substantial mortality under highly stratified conditions in the vicinity of estuarine fronts or strong mixing regimes when freshwater runoff is high. Moreover, our results showed that *A. tonsa* performs better than *A. clausi* in terms of feeding after identical salinity shocks, strengthening the idea of a competitive advantage for *A. tonsa* in environments where animals are prone to face such conditions. Resilience of *Acartia tonsa* to both long-term low salinities and to instantaneous fluctuations indicated by lethal and sub-lethal responses is nearly as high as in *E. affinis* (Von Vaupel-Klein and Weber, 1975). That may help explaining the outstanding ecological success of *A. tonsa* in estuaries and coastal water bodies featuring significant spatial/ temporal salinity fluctuations.

Broad physiological tolerance (e.g., euryhalinity and eurythermy) may favour the spread and establishment of a given species in new habitats (Williamson and Fitter, 1996). Thus, the high haline tolerance of *A. tonsa* likely contributes to its ability to colonize and dominate the estuarine plankton over ample regions of the world. For instance, *A. tonsa* is abundant and among the dominating species in the oligohaline zones of the three largest estuaries in the south-western Atlantic: Lagoa dos Patos (Montú, 1980), Río de la Plata (Cervetto et al., 2006) and Bahía Blanca (Hoffmeyer, 2004), and in Europe *A. tonsa* underwent a profound expansion over the 20th century reaching the Baltic and Mediterranean Seas (Brylinski, 1981), the Black Sea (Gubanova, 2000) and the Caspian Sea (Kurashova and Abdullayeva, 1984).

Results reported in the present study clearly showed that salinity fluctuation is a relevant issue to consider for a better understanding of copepod adaptations to physically changing environments and the consequences of such adaptations for ecosystem functioning. Important aspects to be elucidated in future studies include potential differences in salinity tolerance between sexes and developmental stages, and effects of salinity stress on fertilization and embryo development (see also Lee et al., 2007 and references therein for larval development and rapid physiological evolution induced by salinity changes in *E. affinis*). Ultimately, the physiological capacity of individual species and different life stages to cope with salinity stress may be crucial for how the food web functions — especially in strongly stratified systems — and for how efficiently energy is passed on to higher trophic levels.

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