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Acute and sublethal effects of cadmium on ingestion, egg production and life-cycle development in the copepod *Acartia tonsa*

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ABSTRACT: The acute toxicity of cadmium (expressed as 96-h LC_{50}) to the suspension-feeding copepod *Acartia tonsa* was highly influenced by temperature. The LC_{50}-value was 151 ppb Cd at 13 °C, but only 29 ppb Cd at 21 °C. Salinity influenced the toxicity of cadmium. At 40 ppb Cd the clearance of algal cells *(Rhodomonas baltica)* was reduced at 9 and 17 ‰ S, but not at 25 ‰ S. There was a tendency to reduction of both algal cell ingestion and egg production at cadmium concentrations above 10 ppb, and the reduction was significant above 30 ppb. The development of copepodites and adults was influenced by both salinity (9, 17 and 25 ‰ S) and cadmium (5, 10 and 40 ppb). The general tendency was increasingly longer developmental time at higher salinities and higher cadmium concentrations. The quickest development took place at 17 ‰ S and no cadmium which was probably a consequence of genetic adaptation to intermediate salinity. The slowest development was observed at 25 ‰ S and 40 ppb Cd where the cephalothoracic length in adult copepods was significantly reduced. Adult fertilized egg-producing females developed under all experimental conditions, and it is concluded that the 96-h LC_{50} values were close to the higher sublethal concentrations of Cd which reduced ingestion, egg production and developmental time, but allowed the copepod to accomplish an entire life-cycle.

INTRODUCTION

It has often been stressed that toxicological effect studies on pollutants should not deal only with acute toxic effects (measured as e.g. 96-h LC_{50}). Such studies should be replaced or complemented by studies of sublethal effects on bioenergetic parameters (ingestion, respiration, growth etc.) in adults as well as in younger more sensitive developmental stages, in order to unveil latent long-term effects of pollutants at low concentrations (e.g. Rosenberg & Costlow 1976, Johns & Miller 1982).

Copepods are important links in marine food chains, and their ubiquitous distribution and small size suggest them as a choice for bioassay organisms in pollution studies. The egg production rate of copepods has been found to be a sensitive short-term indicator of sublethal effects with obvious ecological significance, although limited to adults (Reeve et al. 1977). The importance of life-cycle toxicity tests to determine the effects of pollutants has been emphasized by Nimmo et al. (1977). Hitherto, no comparative studies on LC_{50}, sublethal effects on egg production, development, and growth during the life-cycle have been performed on marine copepods.

Since the late 1960's increasing interest has been devoted to cadmium as an environmental contaminant, but studies of its effects on marine organisms are more recent (Møhlenberg & Jensen 1980). In the present work the LC_{50} and sublethal effects of cadmium have been studied in a marine suspension-feeding copepod, *Acartia tonsa,* in relation to ingestion, egg production and life-cycle development.

MATERIALS AND METHODS

The experiments were conducted at the Biological Station at Bøgebjerg, Funen, Denmark. Offspring of copepods originally transmitted as eggs from a laboratory culture of *Acartia tonsa* grown at the Danish Institute for Fisheries and Marine Research were used in the experiments. As egg production in *A. tonsa* is high and constant only during the first few weeks after maturation (Parrish & Wilson 1978), fertilized females that had matured within the previous 1 to 2 wk were

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employed. Copepods to be used in the experiments were reared at known time intervals (1 wk) by transferring 15000 to 40000 eggs sucked from the bottom of a 100 l cultivation tank with all developmental stages of copepods to one of four 100 l cultivation tanks where hatching and subsequent growth and maturation took place. The copepods were reared at 10 to 18 °C in 12 to 20 ‰ S seawater to which phytoplankton algae *Rhodomonas baltica* were added 5 times a week to maintain a mean concentration of about 15000 algal cells ml^{-1}. The cultivation tanks were slightly aerated to prevent sedimentation of the algae. Fertilized females (i.e. carrying a spermatophore) from 3 to 4 wk old cultures were sorted out under a microscope and used for experiments.

Rhodomonas baltica (about 6 µm in diameter) was cultivated at 18 °C and continuous dim light (about 130 µE m^{-2} s^{-1}) in 8 l semibatch-cultures with a concentration of about 10^6 algal cells ml^{-1} ensured by a dilution rate of 25 % d^{-1}. The culture medium was autoclaved natural seawater (15 to 20 ‰ S) enriched with nutrients, but without EDTA or other chelators.

Volume of water cleared of algal cells per unit time (i.e. clearance) was measured by incubating 15 to 20 fertilized copepods in 550 ml screw-cap glass jars containing a suspension of *Rhodomonas baltica* at the desired concentration. By measuring the reduction in algal cell concentration the clearance (F) was calculated according to the following equation of Kiørboe et al. (1982): $F = V/(n \times t) \times \ln(C1 \times C2k)/(C2 \times C1k)$, where V = volume of glass jar; n = number of copepods; t = experimental time; C1 and C2 = algal concentration at times 0 and t, respectively; and C1k and C2k = algal concentration in controls. To each glass jar was added 0.5 ml concentrated algal growth medium to prevent nutrient limitation of algal growth during the incubation time, during which the jars were fixed on a rotating wheel (1 rpm). One d before the experimental measurements were made the copepods were acclimated to the desired algal concentration in the glass jars. At time 0 and 24 h, or every 24 h during incubation periods of 3 d when egg production was measured, samples were withdrawn for measurement of algal cell concentration on an Elzone 80XY electronic particle counting system fitted with a 76 µm orifice tube. The concentration of algae relative to controls without copepods decreased 20 to 50 % during the incubation periods. The mortality was less than 10 % during incubation periods of 3 d.

Food ingestion rate (I) was estimated according to Kiørboe et al. (1982) as the average algal cell concentration (C) times clearance (F): $I = C \times F$; where $C = (C2 - C1)/\ln(C2/C1)$.

Copepod eggs were separated for counting by gently pouring the contents of the glass jars first through a 180 µm mesh sive (which retains the copepods) and then through a 40 µm mesh sieve, which retains the eggs to be counted under a microscope after being dyed with Lugol's iodine solution.

The effect of cadmium on clearance, ingestion and egg production at different salinities and temperatures was studied by adding cadmium (as $CdCl_2$) to the experimental jars with fertilized copepods and 2×10^4 algal cells ml^{-1}. Each experiment run at a desired cadmium concentration was performed with 3 replicate glass jars with added cadmium and 3 control jars without additional cadmium. The significance of the difference between copepods exposed to cadmium and controls was tested by a t-test (Zar 1974).

Dry weight (W, µg) of female copepods was estimated from measurement of the cephalothoracic length (L, mm) in representative specimens using the relation $W = 13.4\ L^3$ (Kiørboe et al. 1985). Mean cephalothorax length was 0.87 ± 0.02 mm corresponding to an average dry weight of 8.82 µg. Assuming a carbon content of 40 % of body dry weight (Parsons et al. 1977), a female *Acartia tonsa* had a content of 3.5 µg C. Carbon content in algal cells and copepod eggs was assumed to be the same as found by Kiørboe et al. (1985), i.e. *Rhodomonas baltica* = 36.7 pg C $cell^{-1}$; *A. tonsa* = 45.7 ng C egg^{-1}.

In order to determine the sublethal concentrations of cadmium to be used in the experiments, the concentration of cadmium that kills 50 % of a *Acartia tonsa* population within 96 h (i.e. LC_{50}) was found at different salinities and temperatures. The LC_{50} experiments were performed by transferring about 20 adult copepods to glass beakers with 250 ml seawater with added cadmium in known concentrations. The copepods were not fed. Dead individuals were counted after 96 h to establish the relation between percent mortality and concentration of cadmium, from which the LC_{50} value could be estimated by means of a probit analysis (Finney 1971).

The sublethal effects of cadmium on growth and developmental time during all ontogenetic stages from hatching to adult fertile stage was studied at 3 salinities (9, 17 and 25 ‰ S) obtained by adjusting the salinity of 16 ‰ S natural seawater with NaCl or demineralized water. Approximately 700 *Acartia tonsa* eggs were transferred to aquaria holding 18 l seawater and containing 2×10^4 *Rhodomonas baltica* cells ml^{-1} and sublethal concentrations of cadmium. The algal concentration was adjusted every second day, and the water changed once every week. Representative samples of the different copepod stages were taken at different times during development by means of a 40 µm mesh sieve to be able to establish the relation between developmental stage and time under the different environmental conditions. At the end of the

experiment the cephalothoracic length of representative specimens from each aquarium was measured. An analysis of variance was employed to test the significance of the difference between treatments. If significant differences ($p = 0.05$) were found, a Newman-Keuls multiple range test was used to determine where the differences occurred (Zar 1974).

RESULTS

Clearance, ingestion and egg production as a function of algal concentration expressed in carbon units are shown in Fig. 1. The few data on clearance at the lower algal concentrations makes it problematic to fit a curve, but apparently the clearance had a maximum at an algal concentration of about 150 ng C ml^{-1}. Ingestion increased sigmoidally with algal concentration and approached a plateau at about 700 ng C $(\mu g\ dw)^{-1}\ d^{-1}$, equivalent to 175 % body carbon per day. Egg production varied linearly with ingestion rate (Fig. 2) and the slope of the regression line indicates a conversion efficiency of 32 % for ingested carbon to egg-carbon. Based on these findings it was decided to carry

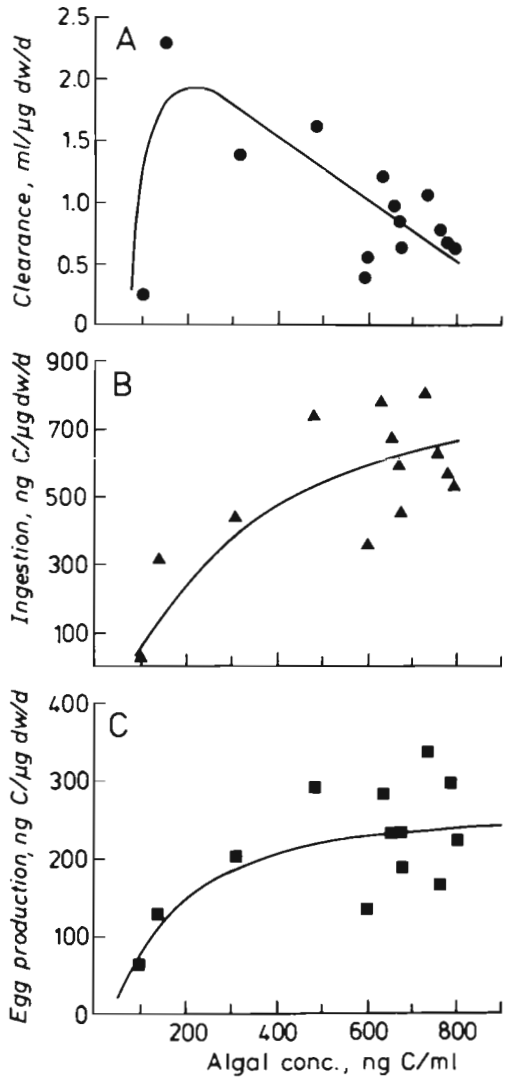

Fig. 1. *Acartia tonsa*. Clearance, ingestion (I) and egg production (G) as a function of algal concentration (C). Each point represents the average of 3 replicates. The lines shown are: (A) fitted by eye, (B) $I = 970 \times e^{-288/C}$ ($r^2 = 0.78$), (C) $G = 287 \times e^{-131/C}$ ($r^2 = 0.65$)

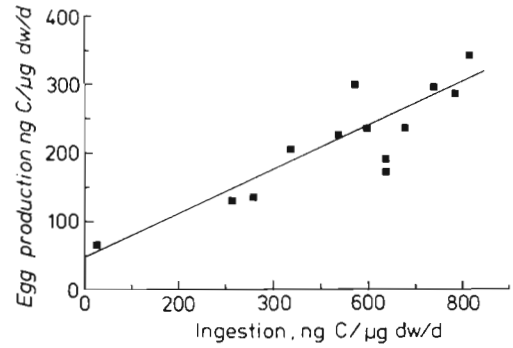

Fig. 2. *Acartia tonsa*. Egg production (G) as a function of ingestion rate (I). Data from Fig. 1. Regression line is: $G = 46 + 0.32 \times I$ ($r^2 = 0.80$)

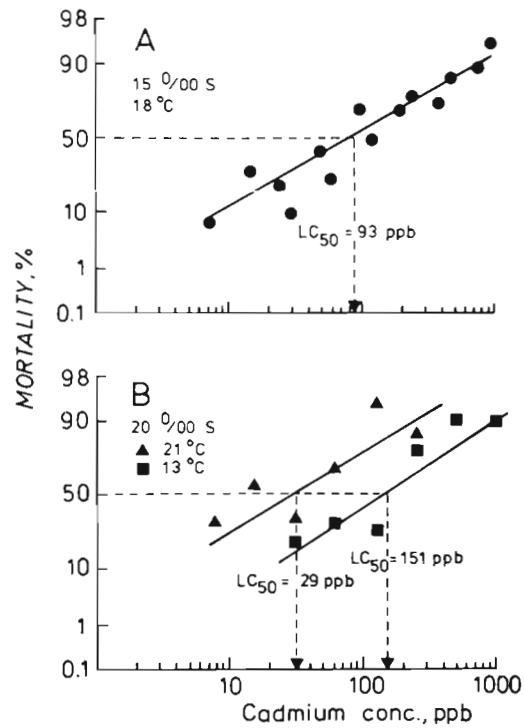

Fig. 3. *Acartia tonsa*. Mortality of copepods (expressed as percentage dead on a probit scale) as a function of cadmium concentration at different salinities and temperatures. The regression lines are shown as well as the estimated 50 % mortality after 96 h exposure (i.e. LC_{50}). The LC_{50} values, with 95 % confidence limits shown in parentheses were: (A) 93 (82–106) ppb Cd; (B) (21 °C) 29 (23–37) and (13 °C) 151 (120–191) ppb Cd

out the cadmium effect studies at an algal concentration of 700 ng C ml^{-1}, equivalent to 1.9×10^4 algal cells ml^{-1}.

The acute toxic effect of cadmium on *Acartia tonsa* was influenced both by temperature and salinity (Fig. 3). The LC_{50} value was 151 ppb Cd at 13 °C, but only 29 ppb Cd at 21 °C. From the LC_{50} values it was decided to run sublethal effect experiments at cadmium concentrations of 10 to 50 ppb.

The effects of 40 ppb cadmium on exponential reduction in algal cell concentration and clearance at different salinities are shown in Fig. 4 & 5. At the lower salinities (9 and 17 ‰ S) reduced clearance could be noticed at 40 ppb Cd whereas no effect was seen at 25 ‰ S.

The effect of cadmium on ingestion and egg production is shown in Fig. 6. There was a tendency for reduction in both of these parameters at cadmium concentrations above 10 ppb, but the reduction was significant above 30 ppb ($p < 0.05$). The conversion efficiency for ingested carbon to egg-carbon (i.e. the gross growth efficiency, since growth in adult females

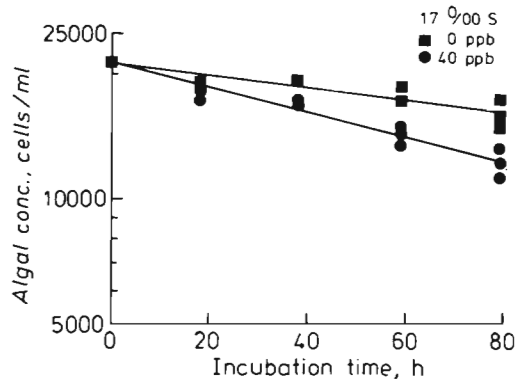

Fig. 4. *Acartia tonsa*. Exponential reduction in algal cell concentration in glass jars with copepods as a function of time in experiments run at 18 °C with 0 and 40 ppb Cd added to the water (17 ‰ S). Regression lines are shown

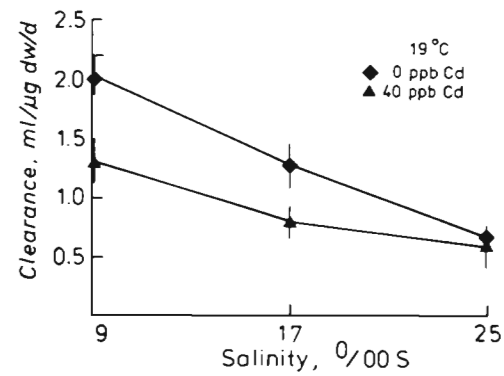

Fig. 5. *Acartia tonsa*. Effect of 40 ppb cadmium on clearance measured during the first day of incubation of copepods in experiments run at different salinities

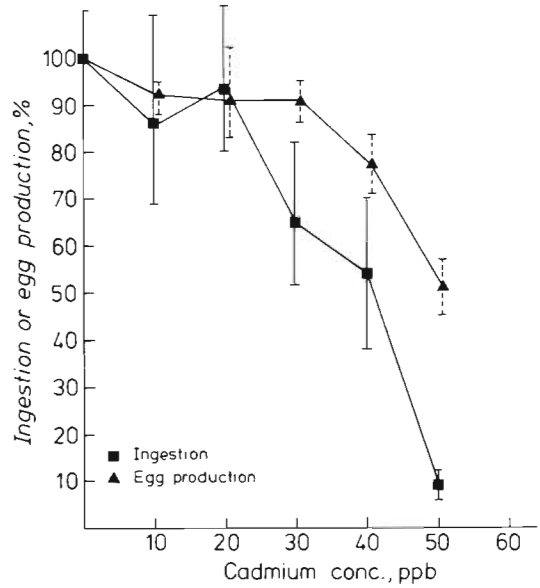

Fig. 6. *Acartia tonsa*. Ingestion and egg production (expressed as percentage of controls) as a function of cadmium concentration in experiments performed at 17 ‰ S and 18 °C. Each point represents the average of 3 replicates. Vertical lines represent the range calculated on the basis of the mean values of Cd-exposed copepods ± SEM in relation to mean values of control ± SEM

is mainly in the form of egg production) was apparently increasing with increasing cadmium concentration (Table 1).

The development of copepodites and adults was influenced by both salinity and cadmium (Fig. 7). The general tendency was longer developmental time at high salinities and high cadmium concentrations. The quickest development took place at 17 ‰ S and no cadmium. The slowest development was observed at 25 ‰ S and 40 ppb Cd. The cephalothoracic length in adult copepods grown under the latter conditions was also significantly reduced ($p < 0.05$) (Table 2). Adult fertilized and egg-producing females developed under all experimental conditions, but the number of eggs laid in 40 ppb Cd experiments performed at 17 and 25 ‰ S were about 25 % reduced. The number of eggs hatched in all experiments with added 40 ppb Cd were about 10 % reduced compared to controls.

DISCUSSION

The clearances measured in the present work (Fig. 1A) are in fairly good agreement with Kiørboe et al. (1985) who found that the clearance in *Acartia tonsa* peaked at a food concentration of about 150 ng C ml^{-1} and decreased at both higher and lower algal concentration levels. Also ingestion, egg production (Fig. 1B,

Table 1. *Acartia tonsa*. Conversion efficiencies for ingested carbon (I) to egg-carbon (G) in copepods exposed to different cadmium concentrations. Mean values ± SEM of 3 replicates in cadmium experiments and 15 replicates of controls

Cd. conc. (ppb)	G/I
0	0.40 ± 0.02
10	0.45 ± 0.02
20	0.42 ± 0.03
30	0.51 ± 0.03
40	0.61 ± 0.06
50	2.06 ± 0.19

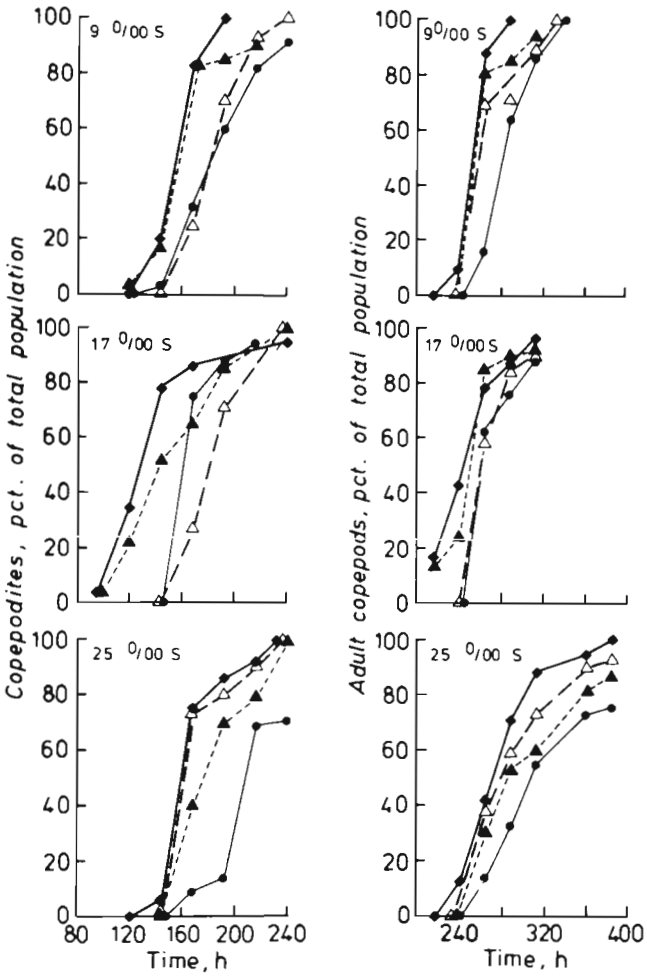

Fig. 7. *Acartia tonsa*. Development of copepodites (CV) and adults expressed as percentage of the total population as a function of time from transfer of eggs to growth aquaria in experiments performed at 18 °C and different salinities and cadmium concentrations. (◆) Controls; (△) 5 ppb Cd; (▲) 10 ppb Cd; (●) 40 ppb Cd

C) and conversion efficiencies for ingested carbon to egg-carbon (Fig. 2) in the present work are in good agreement with Kiørboe et al. (1985) who found for example that the ingestion rate was equivalent to 180 % of body carbon per day (compared to 175 % in this work) and that ingested carbon was converted into egg-carbon with an efficiency of 36 % (compared to 32 % [Fig. 2] and 40 % [Table 1] in this work).

Table 2. *Acartia tonsa*. Cephalothoracic length (mm) in adult copepods grown at 18 °C and different salinities and cadmium concentrations (see also Fig. 7). Values are the mean of 12 measurements ± SEM

Cd conc. (ppb)	9 ‰	17 ‰	25 ‰
0	0.89 ± 0.01	0.89 ± 0.01	0.86 ± 0.01
5	–	–	0.86 ± 0.01
10	0.88 ± 0.01	0.89 ± 0.01	0.88 ± 0.01
40	0.90 ± 0.01	0.86 ± 0.01	0.81 ± 0.01

The time necessary for egg production to come into equilibrium with ingestion rate was measured to be about 1 d by Kiørboe et al. (1985). This explains the phenomenon of apparently increasing conversion efficiencies at increasing cadmium concentrations (Table 1): when ingestion decreases due to the effect of cadmium this gives rise to apparently higher conversion efficiencies because egg production varies with ingestion rate with some time lag. This phenomenon also explains the less pronounced effects of cadmium on egg production compared to the effects on ingestion rate (Fig. 6). The reduced egg production in cadmium-exposed copepods seems to be a consequence of reduced ingestion rate (Fig. 4 & 5).

The acute toxicity of cadmium (expressed as 96-h LC_{50}) on *Acartia tonsa* was influenced by both salinity and temperature. At 15 ‰ S (18 °C) the LC_{50} value was 93 ppb (Fig. 3A), but at 20 ‰ S LC_{50} values were 151 ppb and 29 ppb at 13 and 21 °C respectively (Fig. 3B). The LC_{50} values may be compared with 96-h LC_{50} values of 90 to 337 ppb Cd (10 ‰ S, 20 °C) and 122 ppb Cd (30 ‰ S, 20 °C) found in *A. tonsa* by Sosnowski & Gentile (1978), and 15.5 ppb Cd (15 to 23 ‰ S, 20 to 28 °C) in *Mysidopsis bahia* by Nimmo et al. (1977).

Salinity also influenced the effects of cadmium on clearance (Fig. 5). At 40 ppb Cd clearance was reduced at 9 and 17 ‰ S, but not at 25 ‰ S. The protective effect of high salinity is probably attributable to chloride ion complexation of cadmium. Similarly, a reduced mortality of cadmium in shrimps at higher salinities was correlated with a decreasing concentration of free (toxic) cadmium ions due to cadmium complexation by chloride ions (Sunda et al. 1978). According to Long & Angino (1977) the chloro-complexes account for an increasing fraction of dissolved forms when salinity increases. Free cadmium ions thus gradually decrease

reaching only about 2 % of dissolved forms in 35 ‰ S seawater. If cadmium is taken up by the same mechanisms as calcium, the higher toxicity of cadmium noted at the lower salinities may also partly be due to a higher accumulation rate of cadmium at lower salinities when the calcium concentration is low and uptake accelerated in order to meet calcium demand (Wright 1978).

The higher clearance (Fig. 5) and faster development at intermediate salinities (Fig. 7) is probably a consequence of genetic adaptation to low salinity. The *Acartia tonsa* used was originally isolated from the brackish Øresund, Denmark. This makes it difficult to discriminate between the toxic effects of cadmium in relation to its degree of complexation by chloride ions at different salinities and the effects of different salinities. From the present findings it may, however, be concluded that the acute toxic concentrations of cadmium (expressed as LC_{50} values) on *A. tonsa* are quite close to the higher sublethal concentrations which reduce ingestion, egg production and developmental time, but allow the copepod to accomplish an entire life-cycle. The cadmium concentrations used in our experiments may be compared to a mean of 0.025 ppb Cd in the Danish Sounds and the Kattegat (Magnusson & Rasmussen 1982).

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