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The costs of crest induction for *Daphnia carinata*

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Abstract The effects of notonectid-induced crests on growth and reproduction, and resource allocation to crest construction, moult losses and eggs of *Daphnia carinata* were measured. An attempt was made to elucidate the mechanisms of physiological costs of crest induction for this species. The crested morph of *D. carinata* reached a significantly larger size than the uncrested form. Reproductive output was similar in early instars, but the crested morph produced more eggs in latter broods. Instar duration was longer for the crested morph and age at first reproduction was delayed. Survival was also lower in this form. Crest construction required significant resources (equivalent to 60 eggs over a life time) but evidence is presented that these resources were obtained primarily by re-allocation of available material rather than collection of extra resources. The crested morph allocated significantly more resources to moulting than its uncrested counter-parts. The uncrested morph produced large eggs in early instars and progressively smaller ones in later instars. The crested morph produced only small eggs. The hypothesis is presented that the crest-induction strategy of *D. carinata* involves at least two separate sets of responses, each with its own costs and trade-offs. The first response is production of the crest. The cost of crest production is an increased cost of moulting. *D. carinata* off sets this cost by increasing instar duration and thus age at reproduction. The second response is increased size. *D. carinata* achieves this by reducing the fraction of available resources allocated to reproduction. The cost is lower reproductive output.

Key words *Daphnia* · Crest induction · Energetic costs

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Introduction

Seasonal variation in morphology (cyclomorphosis) has fascinated limnologists for more than a century, and a vigorous debate has ensued in the literature as to its ultimate function (see Hutchinson 1967 for an extensive review). This debate, however, has been largely relegated to historical interest as new insights have indicated cyclomorphosis to be principally related to predator defence. Several studies have shown that the exuberant features reduce the efficiency of invertebrate predators (Dodson 1974; Grant and Bayly 1981; Havel and Dodson 1985; Reynolds and Geddes 1984; Havel 1985; Barry and Bayly 1985; Kenkataraman and Krishnaswamy 1986; Pijanowska 1990), thus providing evidence that defence is the principal role of cyclomorphic change. Jacobs (1987), however, argues that defence against predators may only be one of the functions of the crest.

Temperature (Egloff 1968), temperature and turbulence (Havel and Dodson 1985), food (Wesenburg-Lund 1908; Hebert 1978a, b), turbulence and light (Jacobs 1962; Hazelwood 1966) have all been implicated as proximate triggers of cyclomorphosis. However, none of these factors have been found to produce fully crested individuals. The most potent and unequivocal results have been obtained from soluble substances released by potential predators (Grant and Bayly 1981; Krueger and Dodson 1981; Barry and Bayly 1985; Hebert and Grewe 1985; Dodson 1988a, b, 1989; Dodson and Havel 1988). A comprehensive understanding of the initiating factors is far from complete, but it appears that they may operate in a hierarchy, dominated by direct chemical stimulation, with the other factors acting only as secondary modulators.

Where the production of defensive morphology is triggered by contact with the predator or its metabolite, the term "predator-induced defences" has replaced cyclomorphosis. Predator-induced defences have been reviewed by Havel (1986). More recently, several studies have shown that predators may also cause less obvious changes in growth, behaviour and morphology. Dodson

(1988b, 1989) has demonstrated that both predatory fish and notonectids may chemically affect the size of *Daphnia* at maturity. He has suggested that the daphnids optimise their chances of reproducing at least once by reducing size at maturity. Thus, they have the opportunity to reproduce before reaching a size which exposes them to predation by fish. These changes correspond closely with the optimality models of Lynch (1980).

Several studies have documented modifications in life-history parameters associated with the production of defensive spines or crests in *Daphnia* (Hebert 1978a; O'Brien et al. 1980; Riessen 1984; Barry and Bayly 1985; Havel and Dodson 1987; Jacobs 1987; Ketola and Vuorinen 1989; Walls and Ketola 1989; Riessen and Sprules 1990; Black and Dodson 1990). However, far from producing a clear and consistent picture, these studies describe confusing and often conflicting scenarios. Hebert (1978a) and O'Brien et al. (1980) both reported larger brood sizes in crested daphnids than their uncrested counter-parts. In contrast, Barry and Bayly (1985) found smaller broods in crested *Daphnia carinata*. Jacobs (1967) and Havel and Dodson (1987) found no difference in brood sizes, but the latter authors did record delayed reproduction in the spined morphs. Ketola and Vuorinen (1989) found that a cyclomorphic form had smaller broods initially but produced larger ones in later instars. They also found that the rate of reproduction was retarded. This confusing array of results led Jacobs (1987) to conclude, "All in all, the thesis of a reproductive disadvantage of exuberant forms remains controversial".

When those studies which have examined the effects of crest growth on life-history parameters in *Daphnia* using "longitudinal data" (*sensu* Cock 1966), are examined (Havel and Dodson 1987; Ketola and Vuorinen 1989; Walls and Ketola 1989; Riessen and Sprules 1990; Black and Dodson 1990), one consistent feature emerges – all these studies have found that age at first reproduction increases in the defensive morph. However, no consistent pattern emerges with respect to any of the other aspects of life history which have been studied (age at maturity, brood size, egg size or survivorship, or number of juvenile instars) and the ultimate basis for these costs has, remained a mystery. One reason for this confusion may lie with our ignorance of the fundamental energetics of crest induction.

The goal of this study was to measure the costs of crest induction for *D. carinata* and elucidate their physiological and energetic basis. The study progressed through three stages as follows:

1. Measurement of the effects of predator stimulus (a chemical released by the notonectid *Anisops gratus*) on growth and reproduction of *Daphnia carinata*
2. Measurement of the amount of resources required for crest construction and the effects of crest induction on moult losses and egg size
3. Construction of energy budgets for crested and uncrested *D. carinata*

Materials and methods

A single clone of *D. carinata*, originating in a farm dam near Lara in south-western Victoria, was used in all experiments unless otherwise specified. Cultures were maintained in the laboratory under experimental conditions for several generations prior to the commencement of the study.

Experiments were carried out in a constant temperature room at 20°C, with a 14 h light/10 h dark regime, provided by a bank of fluorescent lights (600 lux). *Daphnia* were grown in Keating's animal medium (DM) (Keating 1985), with minor modifications. Keating's medium was modified by using deionized rather than distilled water, and by not adding a buffer. The pH of the DM was around 6.8–7.0 and remained stable. *Daphnia* were fed non-axenic *Chlamydomonas* sp. *ad libitum*. The *Chlamydomonas* were cultured in Keating's algal medium (AM) (Keating 1985) buffered to pH 8.0, in deionized water. The AM was modified by substituting "Tris" (hydroxymethyl)-aminomethane for the glycolglycine buffer recommended by Keating. Cultures were held in 3- and 5-L erlenmeyer flasks under constant illumination from a bank of fluorescent lights and were maintained in log-phase growth by changing 60% of the solution every 3–4 days. The algae were harvested by gentle centrifugation and cell concentrations determined with a coulter counter.

Daphnia were measured using a stereo-binocular microscope fitted with an ocular micrometer. Three morphometric parameters were measured on all daphnids: body length (BL), total length (TL) and crest height (CR) (Fig. 1).

Growth experiment

This experiment was performed in large plastic containers filled with 2 l of artificial pond water (DM). *Daphnia* were fed *Chlamydomonas* at 2×10^5 cells ml⁻¹. This algal concentration was above the incipient limiting food level for this species of *Daphnia*.

Single specimens of the predaceous notonectid *Anisops gratus* were placed in cages in half the containers. The cages were constructed from 60-ml plastic vials, without lids. The open end of each vial was covered with 110- μ m nylon mesh, sealed using silicon rubber glue. The cultures were placed in the 20°C constant temperature room in a fully randomized design. Pasteur pipettes connected to an air supply system were used to gently aerate the cultures and to reduce settling by algae.

The experimental *Daphnia* were prepared by collecting the offspring of a single female and growing them to maturity in isolation under optimal conditions. After this cohort had produced their first young and brood pouches contained the second batch of eggs, the mothers were randomly separated into two groups. One group were exposed to *Anisops* factor and the other maintained in a predator-free environment. Thus, the experimental animals were exposed to constant conditions throughout their development. Because the eggs of the second brood were laid into the pouch before transfer, complications due to phenotype-specific egg size variation were avoided. Young from the predator cultures were placed in containers with *Anisops* and the others in the controls.

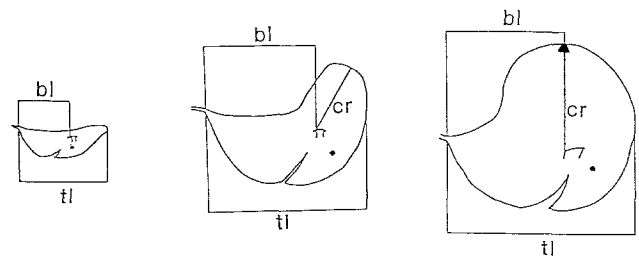


Fig. 1 Main morphometric parameters of *Daphnia carinata* for instars 1–3 (left), 4–8 (centre), 9+ (right). Key: tl total length, bl body length, cr crest height

Initially 20 daphnids were placed in each container. A second group of neonates from each condition was collected for morphometric measurement. At days 2 and 4, three daphnids were randomly removed from each culture, killed, and measured. In this way unnecessary handling of the experimental organisms was avoided at the early stage in life when they may have been more susceptible to injury. At day 6, daphnids were culled, leaving only six animals per replicate for the rest of the experiment. The experiment was concluded after 24 days due to significant mortality among the crested daphnids.

Culture medium was replaced every second day at which time the animals were measured. Neonates were collected daily once the animals matured. The notonectids were fed every morning by adding three of four adult daphnids to each cage. Normally all the food was consumed within an hour. The predator cages were cleaned once every 24 h to prevent the corpses decomposing in the experimental containers.

The intrinsic rate of natural increase (r) was calculated using the formula

$$r = \ln R_0 / T_c$$

where $R_0 = \Sigma V_x$ = net reproductive rate

$T_c = (X \times V_x) / V_x$ = cohort generation time

$V_x = (l_x \times m_x)$

X = age in days

l_x = proportion of original cohort surviving

m_x = age specific fecundity

Crest weight

Crested individuals from the field site were killed by immersion in warm water and, after measurement of BL and CR, the helmets were carefully removed using a sharp scalpel. The crest was dissected in such a way as to leave the remaining carapace in approximately the shape of a typical, uncrested individual. It was not possible to cleanly remove the crests of juvenile daphnids, so only adults were included in the analysis. After dissection, the crests were placed in teflon vials, freeze-dried, and weighed using a Cahn micro-balance to 0.1 μg . The weight of the whole daphnid was calculated using a length-weight regression as fragments were sometimes lost from the body during dissection.

Crest construction

The material required for crest construction may be obtained either by rearrangement of existing material within the body or by the addition of new material to the soma. If the former is correct, the relationship between total body weight and a standard measure of size (in this case BL) should be the same for both morphs. If the latter proposition is correct, the crested morph should be heavier for any given body length. It should be stressed that the hypotheses are not mutually exclusive and crest production may involve both rearrangement of material within the body and the addition of extra tissue.

Regression lines describing the relationship between body length (BL) and dry weight for crested and uncrested *D. carinata* were calculated to determine which of these hypotheses is correct. Healthy, well-fed juvenile and adult animals maintained at 20° C were used for the analysis. From the adults only females bearing freshly laid eggs were chosen. To avoid compounding somatic with reproductive tissue, the eggs were carefully dissected out of the brood-pouch, using a fine needle, before the animals were freeze-dried and weighed using a Cahn micro-balance to 0.1 μg .

Moultng costs

A total of 25 crested and 25 uncrested daphnids of various sizes were removed from stock cultures, their body lengths (BL) measured, and they were then placed individually in plastic vials containing 50 ml of DM artificial pond water and *Chlamydomonas*, *ad libitum*. The animals were maintained in a constant temperature room at 20° C, under fluorescent lighting with a 16-h light – 8-h dark photoperiod.

The vials were checked daily and moults collected as soon as they were shed. The moults were placed individually on pre-weighed aluminium trays and stored at – 20° C until the completion of the experiment. They were then freeze-dried and weighed to the nearest 0.1 μg on a Cahn micro-balance.

Egg size

Crested and uncrested daphnids holding eggs more than 4 but less than 20 h old (equivalent to developmental stage 2 in Green 1956), were collected from well maintained batch cultures at 20° C, killed by immersion in warm water and measured. The animals were then individually transferred to shallow watch glasses containing fresh water. The eggs were carefully removed from the mother and allowed to settle at the bottom of the dish where they could be counted. The eggs were approximately circular, so area was chosen as a correlate of volume. The area of ten eggs from each daphnid was measured. All the eggs were measured when the brood contained less than ten eggs. After measurement, all the eggs were picked up in a Pasteur pipette and transferred to a pre-weighed miniature aluminium boat. When all the animals had been measured, the eggs were frozen at – 20° C, freeze-dried and weighed on a Cahn electro-balance.

Results

Growth experiment

The presence of *Anisops* had a significant effect on growth of *Daphnia* (Fig. 2). Data from days 2 and 4 were analysed using a two-way ANOVA (factors: phenotype and day; dependent variables TL and BL), as this data was collected using destructive sampling. Data from

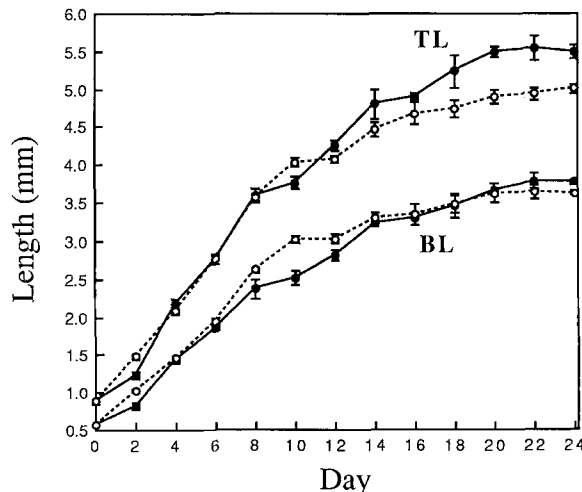


Fig. 2 Growth in terms of total length (upper lines) and body length (lower lines) (\pm SD). *D. carinata* in the presence of *Anisops gratus* (closed circles) or in its absence (open circles)

days 6 to 22 were analysed using repeated-measures ANOVA. Day 24 was excluded because of high mortality. All data points were log-transformed before analysis to remove a positive correlation between the means and variances.

The analyses of data from days 2 and 4 indicated a significant effect of both age and phenotype on BL ($P < 0.001$). The interaction term was also highly significant ($P < 0.001$). Inspection of the means revealed that the uncrested morph was larger on day 2, while on day 4 no significant difference was detectable. There were no significant differences in TL on either day ($P > 0.05$).

The repeated-measures ANOVA indicated no overall difference in BL ($P > 0.05$), however, the interaction term was highly significant ($P < 0.001$). For TL, the crested daphnids were significantly larger in general ($P < 0.01$), although the interaction term was also significant ($P < 0.001$).

The instar duration of the crested morph was longer than for the uncrested form. However, the inter-moult period of both phenotypes increased linearly, with instar number, over the study period. While the instar duration of the crested morph was longer than for the uncrested morph in the early instars, the difference steadily decreased with age. Thus, by the ninth instar, there was virtually no difference between the morphs in the duration of each instar (Fig. 3).

All daphnids became mature in the fifth instar, although all the uncrested specimens produced their first eggs 16–24 h before the crested animals. This was a reflection of phenotype-specific variation in instar duration. In the first two reproductive instars mean brood size was larger for uncrested animals; however in subsequent instars the situation was reversed (Fig. 4). To determine if phenotype-specific differences in reproduction were significant, data were analysed using repeated measures ANOVA (instars 5–8), with number of offspring per instar as the dependent variable. There was no overall significant difference in fecundity between the phenotypes ($P > 0.05$). However, the interaction term was significant ($P < 0.05$), indicating instar-specific variation in the reproductive pattern.

There was a trend for mean brood size of the uncrested morph to be higher in the first reproductive instar (uncrested = 19.7 eggs/daphnid, $SD = 3.48$; crested = 13.7 eggs/daphnid, $SD = 5.88$), although a t -test based on the mean offspring number in each replicate indicated that the difference was not significant ($t = 1.51$, $df = 4$, $P > 0.05$). It is clear from Fig. 4 that the brood size of the crested morph was greater by instar eight.

The mean body length of the uncrested morph was larger at maturity (BL uncrested = 2.62 mm, $SD = 0.03$; BL crested = 2.37 mm, $SD = 0.12$). This was confirmed using a one-way ANOVA, comparing between phenotypes, with the effects of replicates nested within phenotype ($P < 0.001$).

The intrinsic rate of increase (r_0) was 0.44 ($SE \pm 0.001$) for the uncrested morph and 0.39 ($SE \pm 0.03$) for the crested form. A t -test indicated that there was no

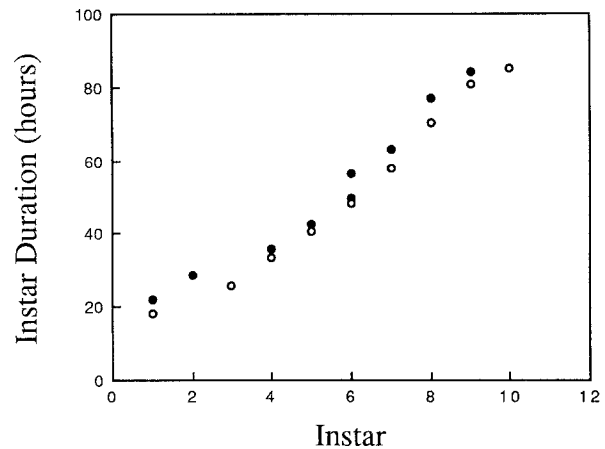


Fig. 3 Instar duration in crested (closed circles) or uncrested (open circles) *D. carinata*

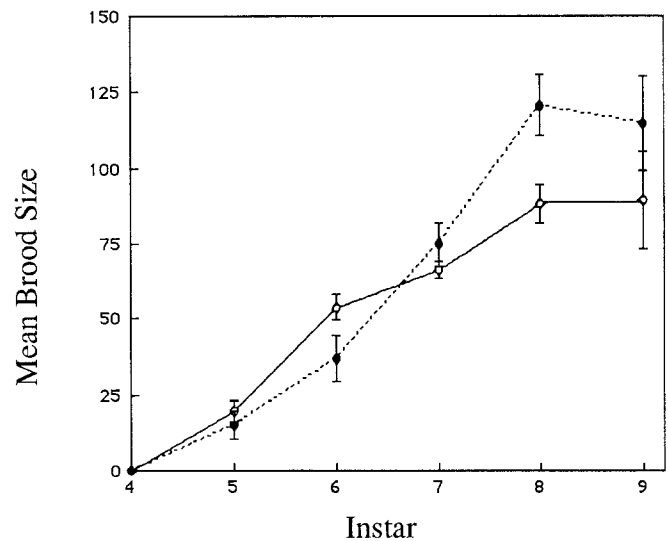


Fig. 4 Plot of brood size (\pm SD) against instar for clone 2 *D. carinata* in the presence of *Anisops gratus* (closed circles) or in its absence (open circles)

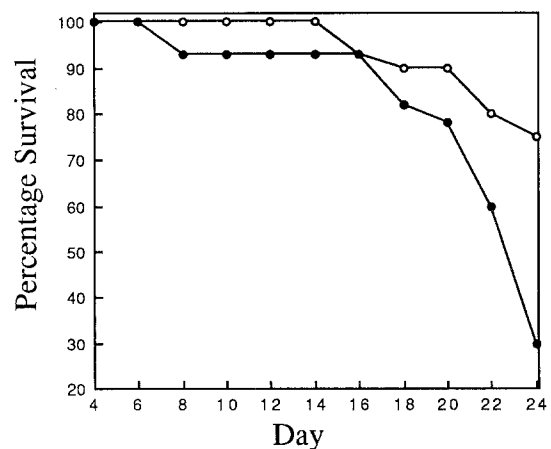


Fig. 5 Percentage survival of crested (closed circles) and uncrested (open circles) *D. carinata* in relation to age (pooling over all replicates)

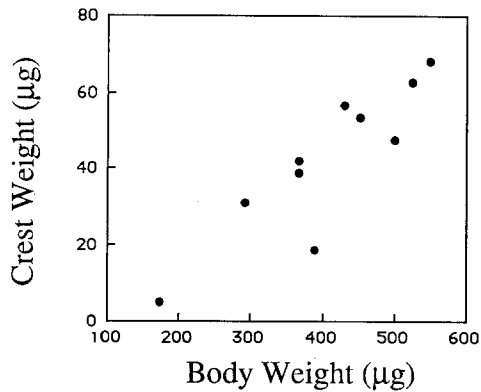


Fig. 6 Plot of crest weight in relation of body weight for *D. carinata*

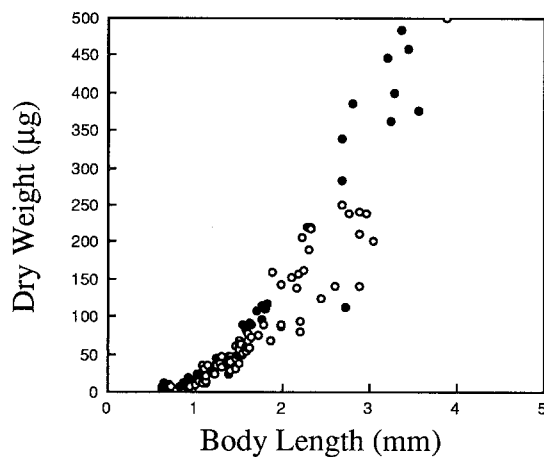


Fig. 7 Dry weight of crested (closed circles) and uncrested (open circles) *D. carinata* in relation to body length (BL)

significant difference at the 0.05 level ($t=2.15$; $df=4$; $P=0.09$).

There were sporadic deaths of *Daphnia* in the cultures containing predators up until day 20, when significant mortality occurred. At this stage there was also mortality in the control cultures; however, survivorship in this morph remained higher than for the crested form (Fig. 5).

Table 1 The absolute and percentage weight of the crest of *Daphnia carinata* for given body lengths or dry weights. The value at a body length of 1 mm was derived by interpolation and at a body length of 4 mm by extrapolation

Body length (mm)	Body weight (µg)	Crest weight (µg)	Crest weight as % of total weight
1	16.38	0.045	0.27
2	108.67	0.47	2.24
3	328.72	25.04	7.62
4	720.96	130.75	18.13

Crest weight

The relationship between body weight and crest weight is illustrated in Fig. 6. The points on the graph were fitted with both linear and power curves. Both curves were highly significant; however, the power function accounted for a higher proportion of the variance in the crest weight. The allometric relationship between crest weight (C) and BL is described in Eq.1 and with total weight (W) in Eq.2.

$$C = 0.04 \text{ BL}^{5.74} \text{ µg dry weight, } r^2 = 0.82 \quad (1)$$

$$C = 0.0001 W^{2.10} \text{ µg dry weight, } r^2 = 0.82 \quad (2)$$

The above regression equation was used to estimate the amount of material in the crest of different sized daphnids (Table 1). This table shows that the fraction of somatic tissue devoted to the crest is small in young animals but increases substantially in large adults. However, as the estimate for juvenile daphnids was obtained by interpolation, the result must be interpreted with caution. When a linear regression is used, the predicted dry weight of the crest in juveniles is much higher.

Crest construction

The length-weight relationships for crested and uncrested daphnids were very similar (Fig. 7). After log-log transformation to linearize the relationship between the variables, linear regressions were fitted to both slopes:

$$\text{Uncrested: } \ln W = 2.78 + 2.70 \ln (\text{BL}), \quad r^2 = 0.92 \quad (3)$$

$$\text{Crested: } \ln W = 2.82 + 2.70 \ln (\text{BL}), \quad r^2 = 0.93 \quad (4)$$

To test for differences in slopes and elevations, a one-way ANCOVA was performed on the data. The analysis indicated that there were no significant differences in either slope or elevation ($P > 0.05$). A common equation describing the relationship between BL and weight was then calculated (Zar 1984):

Common regression:

$$W = 16.38 (\text{BL})^{2.73} \text{ µg dry weight} \quad (5)$$

Moult losses

There were clear differences in size-specific moult weight for the two morphs (Fig. 8). For the purposes of predictive modelling, the data were fitted with a number of different curves. Logarithmic, exponential and polynomial models all accounted for a similar proportion of the variance, however, the logarithmic model was chosen for ease of analysis. The equations are presented below:

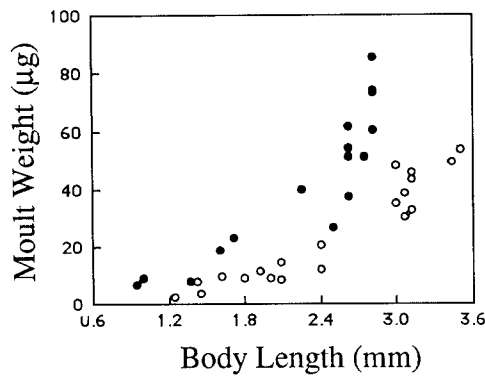


Fig. 8 Plot of moulting weight in relation to body length (BL) of crested (closed circles) and uncrested (open circles) *D. carinata*

Table 2 Absolute and percentage moulting weight for crested (C) and uncrested (U) *Daphnia carinata* of four body lengths

Body length (mm)	Body weight (µg)	Moulting weight (µg)		Moulting weight as % of total weight	
		C	U	C	U
1	16.38	6.7	1.7	41.4	10.7
2	108.67	29.6	11.5	27.3	10.6
3	328.72	70.3	34.7	21.5	10.6
4	720.96	129.8	75.3	18.1	10.5

Uncrested: Moulting weight = $1.75 \text{ BL}^{2.72}$, $r^2 = 0.92$ (6)

Crested: Moulting weight = $6.74 \text{ BL}^{2.13}$, $r^2 = 0.92$ (7)

When the moulting weight is calculated as a percentage of the body weight an interesting difference emerges between the two morphs. For uncrested daphnids, the moulting is around 10% of the weight in all size classes. In contrast to this, the moulting accounts for around 40% of the weight in crested daphnids, but steadily declines with increasing size (Table 2):

Egg size

There was no relationship between maternal body length of brood size and egg weight in crested daphnids ($r^2 = 0$) (Fig. 9a, b). The mean weight of an egg in this phenotype was $2.2 \mu\text{g}$ (SD = 0.36). A correlation may not have been detected because the size range of crested individuals was not as great as for uncrested animals. However, analysis of egg size from field data (Barry 1990) was consistent with this result.

In contrast to the crested morph, there was a negative correlation between maternal body length and brood size with egg weight in uncrested daphnids (Fig. 9a, b). Following log-transformation to linearize the relationship, regression equations were calculated relating maternal size, fecundity, and egg weight (Eqs. 8–10). As maternal size and fecundity were closely corre-

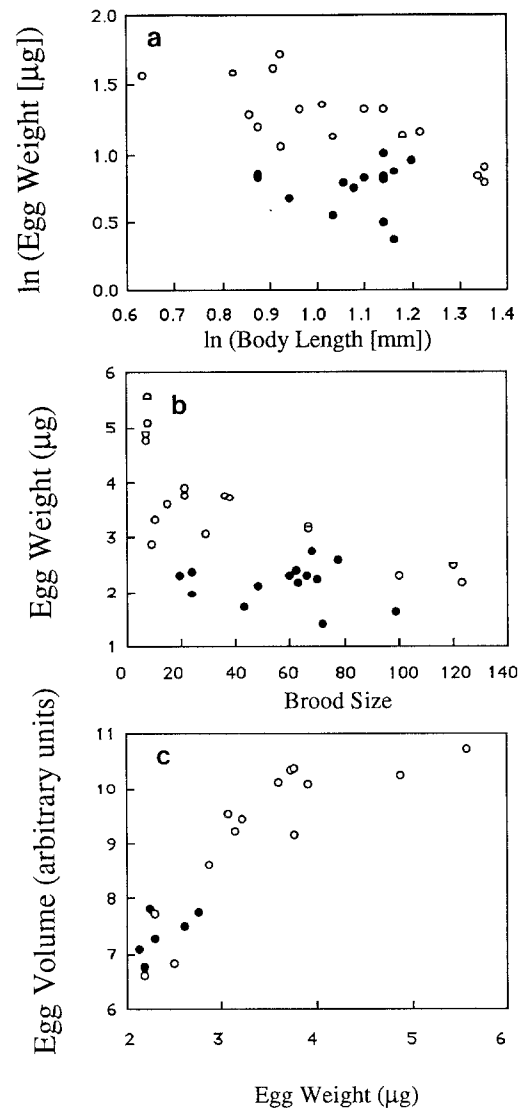


Fig. 9 a Plot of egg weight against body length (BL). b Plot of egg weight against brood size. c Plot of mean egg volume against mean egg weight

lated with each other, the multiple regression equation did not significantly increase the power of prediction.

$$E = 10.12 \text{ BL}^{-1.014} \quad r^2 = 0.585 \quad (8)$$

$$E = 6.84 F^{-0.209} \quad r^2 = 0.635 \quad (9)$$

$$E = (7.21 \times \text{BL}^{-0.118}) \times F^{-0.186} \quad r^2 = 0.636 \quad (10)$$

where E = egg weight (μg); BL = body length; F = number of eggs

When the number of eggs per brood is multiplied by the average egg weight, the reproductive effort (RE) can be calculated in terms of dry weight (i.e. $\text{RE} = E \times F$). For the purpose of modelling growth in *Daphnia*, it is useful to be able to predict brood size from body length and reproductive investment (Equation 11).

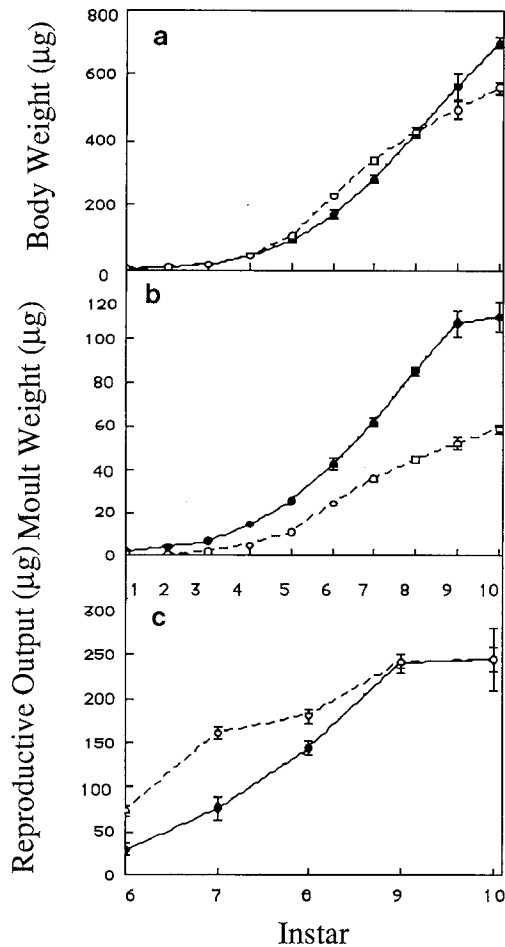


Fig. 10a–c Instar-specific growth of crested (closed circles) and uncrested (open circles) *D. carinata* **a** dry weights (\pm SE), **b** moult losses (\pm SE), **c** reproductive output (\pm SE)

$$F = 0.104 \times BL^{1.423} \times RE^{0.895} \quad r^2 = 0.973 \quad (11)$$

To determine the relationship between the weight and volume of the eggs, the areas measured with the image analysis system were converted to volumes: $V = 4/3 \times A^{3/2} \times \pi^{1/2}$ (where V = volume and A = area). There was a positive correlation between egg weight and egg volume (Fig. 9c). When the data from crested and uncrested animals were pooled the relationship is described by Eq. 12.

$$V = 4.713 E^{0.59} \quad r^2 = 0.820 \quad (12)$$

Energy budgets

To gain a greater understanding of the physiological and energetic factors regulating variation in life-history associated with crest induction the results of the growth experiment were replotted in terms of dry weight per instar (Fig. 10a). Allocation of resources to reproduction was calculated by assuming that each egg of a crested daphnid weighed 2.2 μ g. Egg weights for uncrested

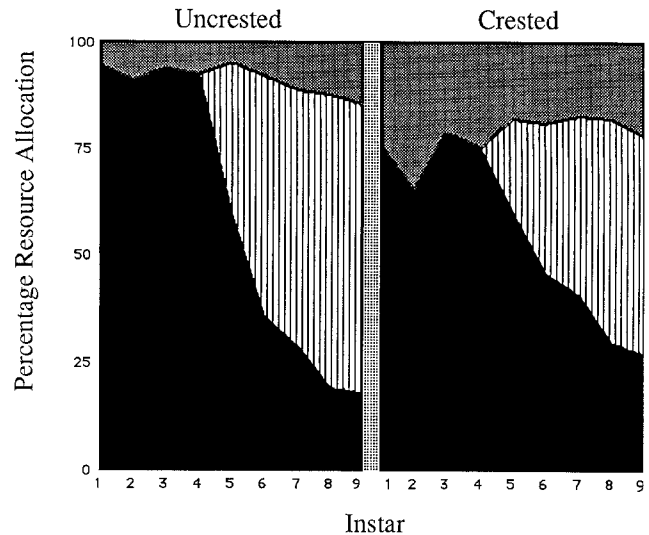


Fig. 11 Relative allocation of resources to growth, mouling, and reproduction in each instar, for crested and uncrested *D. carinata*. Key: black somatic growth, stripes reproductive output, spotted moult loss

daphnids were calculated using Eq. 10 (Fig. 10c). Allocation of resources to mouling was calculated using Eqs. 6 and 7 (Fig. 10b).

When growth of *D. carinata* is plotted in terms of dry weights and instars it is clear that both morphs had very similar development rates until the end of the fourth instar. Beyond this point uncrested animals temporarily exceeded the size of the crested specimens; however, by instar nine the crested specimens were larger. Instar-specific allocation of resources to mouling was much greater for the crested morph than for the uncrested. The uncrested morph devoted more to reproduction than the crested form in the early instars, however, absolute effort converged to the same point by the eighth instar (Fig. 10c).

Somatic growth, moults, and reproduction are the three principal uses for assimilated matter (excluding respiration). By pooling them together it is possible to determine relative instar-specific resource allocation strategies for each phenotype (Fig. 11). The crested morph devoted more resources to mouling than uncrested specimens in each instar. However, it also allocated a greater proportion of available resources to growth, and consequently less to reproduction. The fraction of energy devoted to reproduction by the crested morph was lower, both in absolute terms, and in relative terms, after accounting for additional moult expenditure.

Discussion

Crested daphnids attained a significantly greater total length than their uncrested counterparts. In contrast, Havel and Dodson (1987) found that *Chaoborus* inhibited growth of *D. pulex* and that the crested form

was ultimately smaller than the uncrested morph. Variation in the mode of predation may well account for this discrepancy. Notonectids appear to prefer prey in the mid-size range (Zaret 1980; Reynolds and Geddes 1984), while *Chaoborus* predation is usually restricted to juvenile animals. This suggests a distinct advantage for *D. carinata* faced with *Anisops* in attaining a large size in minimal time, but no corresponding benefit for *D. pulex* when confronted by *Chaoborus*.

The size attained by *D. carinata* in these experiments was exceptional when compared to other species of *Daphnia*. Hebert (1978a) records *D. cephalata* as the largest species, with a mean maximum carapace length of 5.3 mm. He records the largest *D. carinata* (*D. magniceps* Hebert) at 4.7 mm. The maximum size attained in the present experiment for a crested animal was 5.9 mm and for an uncrested individual 5.2 mm. The sizes attained were not artifacts of artificial culture conditions, as specimens at the field site Lara frequently grew to a length (BL) of over 5.0 mm.

Crest induction coincided with a slight reduction in egg number in the first two reproductive instars although there was considerable variability in this factor. In later instars daphnids actually produced larger broods; a pattern also recorded, although not explained, by Ketola and Vuorinen (1989). Crested daphnids were smaller at the age of first reproduction. This result is in congruence with the findings of several other studies (Havel and Dodson 1987; Ketola and Vuorinen 1989; Walls and Ketola 1989; Black and Dodson 1990).

Growth in cladocerans may be broken into two components: instar duration and moult increment. Instar duration was longer for crested specimens. Similar observations have been made in almost all studies which have used a life-table approach in the investigation of cyclomorphosis (Havel and Dodson 1987; Ketola and Vuorinen 1989; Riessen and Sprules 1990; Black and Dodson 1990). The principal consequence of increasing the inter-moult period is to delay the age at first reproduction and thus decrease the maximum potential rate of population increase. Although there was no significant difference in the intrinsic rate of increase the value was close to significance. The low number of replicates in this experiment may account for the lack of significance. In another set of experiments using a morph with a larger crest Barry (1990) found a very clear difference.

Mortality was higher in the crested phenotype. This may represent a genuine cost of crest production in daphnids, or it may be a laboratory artifact. Several other studies have reported higher mortality in predator induced phenotypes (Barry and Bayly 1985; Ketola and Vuorinen 1989); although Riessen and Sprules (1990) found no consistent pattern, and no reports of increased mortality could be found in cases on non-predator (e.g. temperature, turbulence) induction. Black and Dodson (1990) found higher mortality in only one experiment but not in another two. It is significant that Ketola and

Vuorinen found higher mortality in apparently non-cyclomorphic *D. magna*, when exposed to predators.

In contrast to the results of Jacobs (1962), who found that the crest represents an insignificant fraction of total energy expenditure, the crest in *D. carinata* cannot be regarded as energetically inconsequential. Over a life span, a crested *D. carinata* must spend the equivalent of almost 60 eggs on structural tissue alone to construct a large helmet.

The production of a crest in *D. carinata* was associated with more complex changes in the shape of the rest of the body. There was often a reduction in body width in the early instars (although in one morph, "*D. magniceps* Hebert", the crest is formed over the mid-section). Thus, the helmet may not require additional tissue but may be constructed by a rearrangement of existing material within the body. Material for the crest may not only be drawn from structural tissues but other sources could be used. In particular, crested animals were observed to have lower lipid reserves, although this was not quantified. The fact that there were no significant differences in either the slope or elevations of the phenotype-specific length-weight regressions supports the hypothesis of resource reallocation as opposed to additional material, however, the hypotheses are not mutually exclusive and both sources may have contributed.

In the uncrested morph, approximately 10% of dry weight was contained in the moult. This is similar to the 6% to 12% reported by Lynch (1989) for *D. pulex*. Lynch found the fraction increased substantially with size, whereas it remained constant in *D. carinata*. In the crested form, the fraction of mass accounted for by the crest actually decreased with increasing size from around 40% down to 18%. It is clear, however, that the additional moult loss borne by the crested specimens must be a burden affecting the maximum potential growth rate of the morph.

Analysis of egg weights indicated that, under conditions of high nutrition, the crested morph produced eggs which were smaller than those of the uncrested morph. Eggs of the crested morph normally weighed around 2.2 µg. Eggs of the uncrested morph varied from around 2 µg up to 6 µg dry weight. Interestingly, the mean size of the eggs of the uncrested morph became progressively smaller as the animals became larger and produced bigger broods. Thus, the uncrested morph produced large eggs in the early reproductive instars and progressively smaller ones as it aged, while the crested morph produced only small eggs. It is not known if the size of the eggs in the uncrested daphnids changed in response to some pre-programmed ontogenetic plan, or was a reflexive response to larger brood size. Large animals in cultures with poor nutrition appeared to produce a few large eggs, however this was not measured quantitatively.

The clear advantage of reducing egg size is that more offspring may be produced with less resources, however,

the consequences of such a reduction in effort per offspring may be far-reaching and complex: many theoretical works on life-history and resource partitioning have suggested that total reproductive effort can be treated separately from effort per offspring (Stearns 1976; Charlesworth 1980), however, several recent papers have shown that interactions between the two may be complex and interpretations frequently conflict (Lynch 1980; Dorazio and Lehman 1983; Winkler and Wallin 1987).

Variation in the quantity of resources devoted to each egg may have two effects on the offspring. First, a positive correlation between egg size and neonate size has been noted (Green 1956). Second, the quantity of stored lipids in neonates increases with increasing egg mass (Tessier and Goulden 1982). These factors may often be coupled, as Tessier and Consolatti (1989) found a positive correlation between size at birth and weight at birth. Several studies have demonstrated that increasing investment per egg can have a positive effect on the fitness of the offspring. In fact Lynch and Ennis (1983) state that "...slight differences in parental investment at the egg stage alone can have profound and long-lasting effects on the genome expression in these organisms." The effects may, however, be dependent on the food level. Lynch and Ennis (1983) found that lowering egg weight had no effect on energy intake at a high food level, but a 22% increase in egg mass enhanced intake by 100% under conditions of food limitation. Resistance to starvation may also be greatly enhanced by increasing egg weight, and thus increasing lipid reserves in the neonates (Tessier and Goulden 1982; Tessier et al. 1983; Tessier and Consolatti 1989).

For several species of Cladocera, reduction in egg or neonate size may be an adaptive strategy to avoid size selective predation. Of particular relevance is the work of Dodson and Havel (1988), who found that *D. pulex* reduced size at maturity as a means of becoming reproductive before encountering intensive predation by *Notonecta hoffmani*. There is no evidence, however, to suggest this for *D. carinata*. Even though body length at maturity was smaller for crested daphnids, total length remained the same (although the effect of the smaller eggs on the next generation of crested animals is not known). In the field, size at maturity was positively correlated with density of notonectids, however such an association is not strong evidence for causality. The best argument against adaptation is the data on size-selective predation by *Anisops*. Both Reynolds and Geddes (1984) and Barry and Bayly (1985) have shown that young daphnids as small as 2 mm (i.e. normally below the size at first reproduction) may be subject to intense predation by adult notonectids.

On the basis of the available evidence it is not possible to determine the mechanism underlying the switch to smaller egg size in the crested daphnids. The switch may be either a separate strategy, distinct from crest induction, adopted by *D. carinata* or may be a "passive" physiological response to other changes in growth and

metabolism. The end result is, however, a larger brood size for a given reproductive allocation, and thus a higher potential rate for population increase. This may help to compensate of losses to predators and for an increased age at first reproduction.

Another factor which may influence the egg size is the shape of the brood pouch. The brood pouch of the crested morph was frequently observed to be smaller than that of the uncrested morph, although this was not quantified. It is possible that structural considerations necessary for the re-allocation of material for crest construction limit the maximum size of the brood pouch in this morph. Thus, it would be advantageous to reduce the size of the eggs and thus increase the carrying capacity of the pouch. However, in at least one morph of *D. carinata* (*D. magniceps* sensu Hebert), the crest is sited above the mid-region of the body. Thus, in this morph there is no limitation on brood space in the crested morph. Similarly, the size of the brood pouch in large crested adults is often larger than for the uncrested morph.

On the basis of the energetic analysis I would argue that crest induction involves at least two sets of trade-offs; one involving moult losses and instar duration and the second between growth and reproduction. These sets of trade-offs, although related, may actually represent two separate strategies employed by the daphnids. Changes in egg size, associated with the crest induction, may represent a third response.

The crest is the major defensive weapon in the daphnid armoury when confronted by notonectid predation. However, it appears that the construction of the crest incurs additional expenditure on the moult. Nevertheless, when growth data was plotted in terms of instars, there was little if any significant difference in the instar-specific growth increments in juvenile daphnids, the length of instars in the crested morph was longer. The effect of increasing instar duration was to prolong the juvenile period, and to thus delay age at first reproduction.

During the first three or four instars there was no significant phenotype-specific variation in the growth increment (i.e. $W_{\text{instar } n} - W_{\text{instar } n-1}$) (Fig. 10a). However, compared to the uncrested daphnids, the crested morph required an additional total outlay on moult material of 14.4 μg dry weight during this phase of development. At the rates of production observed during the growth experiment this would require an additional 11.85 h.

This is close to the observed difference between morphs of approximately 9 hours for completion of the first three instars. The crested morph has increased the inter-moult period to obtain additional material required for the moult.

An increase in instar duration and the consequent delay in maturity is the most commonly reported 'cost' in a variety of species of *Daphnia* (Havel and Dodson 1987; Riessen and Sprules 1990) associated with crest induction. It is probable that the additional expenditure on the moult is responsible for prolonged instar dura-

tion in these cyclomorphic species, however this must await experimental confirmation.

The trade-off between instar duration and moult loss was not, however, completely balanced. Consequentially, the typical morph was larger at first reproduction. Because assimilation is proportional to body size, net production was also greater in this morph. Thus, the uncrested daphnids not only devoted a larger fraction of resources to reproduction than the crested specimens, but they also had a larger total amount available for this purpose.

Although size and age at first reproduction are widely recognized as important, indeed critical, characteristics in the life-history of cladocerans (Lynch 1980; Dorazio and Lehman 1983), the post-maturation strategy may also be extremely important in determining the ultimate success of a species or morph. Reproductive effort in the crested morph was lower than for the typical form. As a direct result, they were able to attain a larger size and thus increase their net production. This, in turn, allowed the crested daphnids to devote the same amount of resources to reproduction as the uncrested ones (although as effort per offspring was lower they actually produced more eggs in later instars).

Notonectids are size-selective predators and there is good evidence to suggest that the largest daphnids are less vulnerable to capture (Barry and Bayly 1985; pers. obs.). *Anisops* is almost never successful in trying to capture a large crested daphnid. Thus, there may be a strong selective advantage for daphnids exposed to notonectids to grow to beyond the size range of their predators. I would argue that this is a separate strategy, distinct from crested, taken by the daphnids as a defence against the notonectids. It is not a direct or necessary consequence of crest induction with its associated costs and trade-offs, but a separate mechanism for avoiding capture. This is not to say, however, that the two responses are not closely correlated.

A reduction in the size of early broods will clearly lower the intrinsic rate of increase for the population. Although the crested strategy allowed the large adults to balance the reduction in reproductive effort by an increase in absolute production in later instars, they were still at a disadvantage in terms of total output. In the field, however, crested daphnids often reached very large sizes (BL = 4.5–5.0 mm; TL = 7.0–8.0 mm). These specimens, giant “battleships” of the pond, could produce enormous broods during periods of abundant food, and may have been able to counter-balance the reproductive advantages of uncrested phenotypes.

Defence against *Anisops* in *D. carinata* therefore involves at least two sets of responses, each with corresponding trade-offs. These trade-offs are undoubtedly detrimental to the maximal reproductive potential, however; the amount of which they incur demographic costs to the crested morph will depend on the environmental framework in which they occur. In its natural environment in south-eastern Australia *D. carinata* may inhabit either permanent or temporary bodies of water.

Ephemeral habitats typically dry out during summer or autumn and fill during late winter or spring. The first *Daphnia* to hatch from ephippia during the spring frequently find a relatively benign environment, free of predators and often high in food. In such an environment maximizing the rate of reproduction is probably the most effective strategy. Following this initial period where *Daphnia* may colonize the pond relatively free of predation *Anisops* migrate to the pond and may attain high densities by late summer through immigration and reproduction (pers. obs.). In such conditions, particularly if the daphnid population is relatively stable or declining, the value of early reproduction to overall fitness may be reduced or offset by larger broods later in life.

In more permanent environments the density of the *Anisops* population may remain high all year. The field site at Lara in southern Victoria where the clones were originally collected was a semi-permanent body of water. At this pond the density of the *Anisops* population peaked during winter and decreased during spring as the pond volume increased (and presumably because of emigration to ephemeral habitats) (Barry 1990). In this case the population of *D. carinata* was faced with an entirely different set of conditions. These observations suggest that the costs of crest production may be patch-specific and any satisfactory explanation for the response of *D. carinata* to the presence of *Anisops* must account for life-time reproduction and growth patterns.

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