

REPRODUCTION AND SEXUAL MATURATION OF THE SCAVENGING DEEPWATER ISOPOD *NATATOLANA BOREALIS* (LILLJEBORG) FROM WESTERN NORWAY

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The scavenging deepwater isopod *Natanolana borealis* (LILLJEBORG) was caught off Bergen on the West coast of Norway in baited traps. *N. borealis* attained sexual maturity from 6-7 instar. The oostegites were formed in a biphasic parturial-ecdysis. Four marsupial instars were identified and described. The marsupia of the laboratory breeding females contained from 23 to 77 eggs, while the ovaries contained from 28 to 120 oocytes. The time needed for the development of the brood in the laboratory was estimated to be 3-4 months. Breeding females were not caught in the traps. Reproduction took place throughout the year with varying intensity. After the brood release the surviving females went into an intergenerative phase of breeding. The females could have 1-2 broods during lifetime.

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KEYWORDS: *Natanolana borealis*; scavenger; isopod; reproduction; sexual maturation.

INTRODUCTION

The scavenging isopod *Natanolana borealis* (LILLJEBORG) is common off the coasts of Southern and Western Norway from approximately 20 m to 500 m water depth. It occurs rarely in catches made by dredges, sleds, and grabs, but it is caught in vast numbers in baited traps on soft-bottom localities. The limited knowledge of the reproductive biology of *N. borealis* is related to the lack of breeding females in such traps. Captures of breeding females have been reported only occasionally. G.M. Spooner noted breeding in *N. borealis* from February at Plymouth (PLYMOUTH MARINE LABORATORY 1957). WONG & MOORE (1996) caught one manca-brooding *N. borealis* in April 1993 and mancas from February to August 1993 in Loch Fyne, Scotland. My study on *N. borealis* is based on collections from two locations in the vicinity of Bergen, Western Norway in 1977-1978 and 1993-1995. The intention of this study is to examine the reproductive biology and the sexual maturation of *N. borealis*.

MATERIAL AND METHODS

N. borealis was collected monthly in baited funnel traps positioned in Skogsvåg, Raunefjorden (60°16' N, 05°06' E) at 90-100 m depth from September 1977 to November 1978. Raunefjorden is connected to Korsfjorden at Lærøysund with a sill depth of 150 m (HELLE 1975). These waters are highly influenced by the adjacent coastal water. *N. borealis* was also trapped monthly at 80-90 m depth from January 1993 to December 1995 in Osterfjorden, at Raknesvåg (60°34' N, 05°26' E). The interconnections of the estuarine basin system from Osterfjord to the ocean are quite deep (HELLE 1978). The deepest

passage to the coastal water is a narrow one at Hjeltesund with a sill depth of 170 m. No other sound provides a free passage deeper than 40 m. Northerly summer winds induce upwelling of denser water to shallower depths, which can spill over the sill and intrude into the fjord basin.

A mesh-walled, cone-shaped trap with a funnel at the top and a PVC tube with a funnel in one end provided samples from Skogsvåg. The trap used at Raknesvåg was a compact metal cylinder with mesh surrounding the funnels (JOHANSEN & BRATTEGARD 1996). The smallest diameter of the funnel openings was 16-18 mm, about twice the width of the largest isopods. The catches of the monthly collections were sorted and frozen before examination. Living animals were transported in a closed plastic container filled with sea water to the laboratory. The length of the first pleopod of *N. borealis* was used as a measurement of size and measured under a binocular microscope with a micrometer eyepiece to the nearest 0.04 mm. The flexible body of *N. borealis* made total length an inaccurate size measurement. Measurements of pleopod length was more accurate and could also be measured on shed moults. The number of articles on the flagellum of the first antenna was used to identify the postmarsupial instars. Sex was determined from postmarsupial instar 4 by the appendix masculinum on the second pleopod and genital papillae on the ventral integument of the seventh thoracic segment of the males.

The development of eggs was monitored on regularly fed *N. borealis* kept in 30 x 60 x 30 cm aquaria with running sea water. The aquaria contained both males and females. About 200 *N. borealis* were collected at Skogsvåg in February 1978 and the development of oocytes was followed in an aquarium with less than 1 cm sediment with grain size 1-2 mm. The sticky and soft oocytes were counted in distilled water. The development from oocytes in the ovary to marsupial brood was followed in ca 100 *N. borealis* maintained in an aquarium filled with 10-15 cm sediment. The sediment consisted of 78.6 % of grains < 0.25 mm. Both the sediment and the animals were collected at Skogsvåg in November 1977. The development of the marsupial brood was

observed from March 1979 in cylindrical 8 cm diameter and 6 cm high mesh-coated beakers put in running sea water. The different marsupial instars were kept in separate beakers. Eggs which were attacked by fungus were removed by a suction pipette.

Chi square analysis with a priori hypothesis of 1:1 sex ratios were performed in 2 x 2 tables with a Yates correction factor (SCHEFLER 1969). Linear or curvilinear regressions could be used to study relative growth (LOVETT & FELDER 1989). Linear regressions fitted relative growth of *N. borealis* best.

RESULTS

Reproductive development

The ovaries are two long parallel organs situated above the gut. The development of the oocytes in the ovary was separable into four stages. Non-distinguishable oocytes were defined as stage 0. Stage 1 consisted of small white or transparent oocytes. Stage 2 consisted of yellow dorso-laterally situated and medium-sized oocytes. Stage 3 consisted of large yellow oocytes filling a large part of the female thorax. When females with little food in the gut were held against a light source, the oocytes of stage 2 and 3 could be seen through the transparent ventral thoracic integument. The development of the oocytes from stage 0-1 to 3 in the laboratory took about three months at temperatures of 5-7° C in an aquarium with less than 1 cm sediment. During the period from November 1977 to May 1979, 28 females with a marsupium and oocytes of stage 3 were found in the laboratory in aquaria with little sediment. None of these females succeeded in transferring the oocytes from the ovary to the marsupium. Oocytes were, however, transferred to the marsupium in 17 of *N. borealis* in an aquarium with 10-15 cm sediment. Both females and males were present in both types of aquaria. The presence of plentiful amounts of sediment seems to be important in the deposition of eggs in this species.

The following observations indicate that the marsupium was fully developed during the parturial-ecdysis. Females which died during the moult prior to egg laying, had fully developed oostegites only on the posterior thoracic segments and lacked oostegites on the anterior thoracic segments. The shed posterior cuticula did not have oostegites.

The gonopores were not observed on females prior to the marsupial instar. The marsupium of *N. borealis* consisted of five pairs of overlapping oostegites and a caudal pouch with a setosed margin. The caudal pouch was formed by an invagination of the ventral integument extending from the seventh pair of pereopods and forwards. The

mouthparts were partially covered by the first pair of oostegites and the lamina vibrans on the maxillipeds. Lamina vibrans occurred only on the maxillipeds of females with oostegites (HANSEN 1890, tab. 1, fig. 11). He suggested that the first pair of oostegites and the lamina vibrans on the maxillipeds created a waterstream, which passed the eggs. This anatomical configuration did not prevent the incubating female *N. borealis* in the laboratory from eating. Feeding activity, however, resulted in the expulsion of the marsupial brood. The physical ability of the incubating females to feed was also demonstrated from occasional catches of females with empty marsupia in the traps. Scavenging is, however, not a normal activity for incubating females in the field, since they were never caught in the traps in situ.

Four marsupial instars of *N. borealis*, each defined as a period confined by membrane loss or ecdysis, were discovered. The marsupial instars A (egg), B (embryo), C (larva), and D (first manca) are shown in Fig. 1. The broods found in the 17 laboratory breeding females were in marsupial instars A, B, and C.

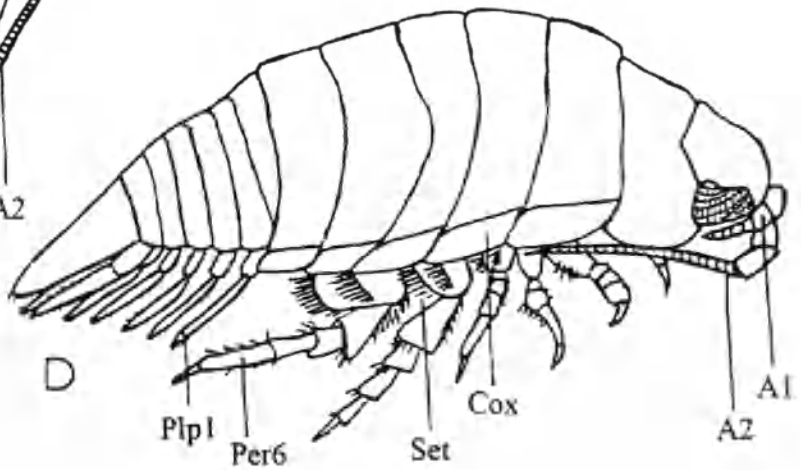
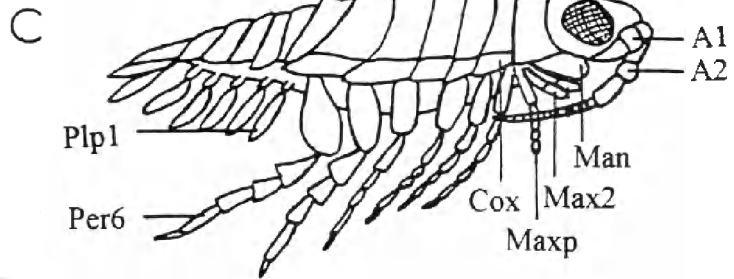
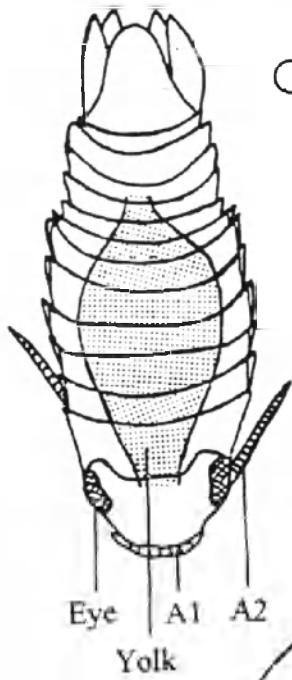
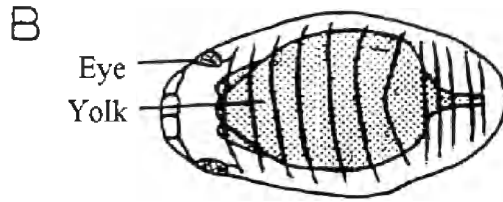
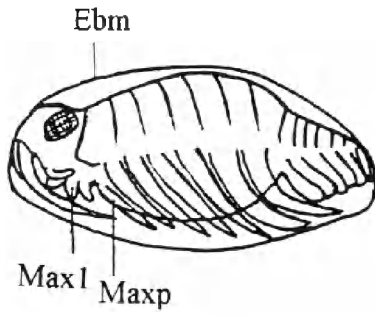
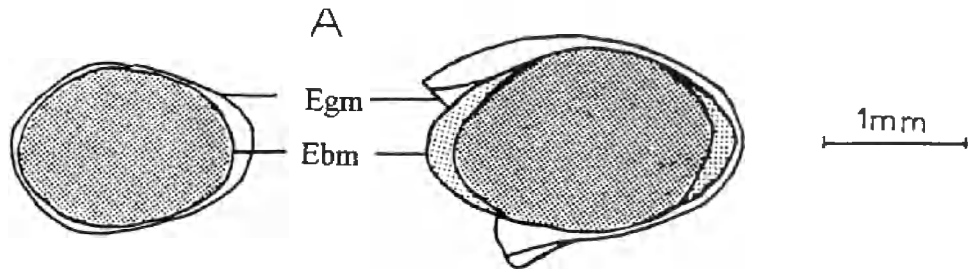
In star A. Two membranes and light yellow yolk. The size of eggs was ca 1.5 x 1.75 mm measured on alcohol preserved specimens.

In star B. The egg membrane was lacking. The yolk was still present. The eyes of the embryo were light brown. The pigmentation started to develop dorsally in this instar. The total length of the embryos were 2.2-2.5 mm measured on alcohol preserved specimens.

In star C. The embryonic membrane was lost. The yolk could still be seen. The larva was unable to swim but could move the body and the pleopods. The pereopods lacked setae. The eyes were dark brown and the body became brown. The articles on the flagellum of the first antenna were not distinguishable. The size of the first pleopod was 0.6 mm.

In star D. After the first moult it became instar D which was identical with postmarsupial instar 1. The yolk could not be seen. The pereopods were setose. Five articles on the flagellum were observed on the first antenna. The seventh pereopods were lacking (first manca). These young were capable of swimming. Cannibalism was observed. The average length of the first pleopod was 0.80 mm (SD = 0.03, n = 20).

Fig. 1. The marsupial instars A-D of *N. borealis*. Abbreviations: Egm, egg membrane; Ebm, embryonic membrane; Yolk, yolk sac; A1, first antenna; A2, second antenna; Plp1, first pleopod; Per6, sixth pereopod; Max1, first maxillae; Max2, second maxillae; Maxp, maxilliped; Man, mandible; Set, setae; Cox, coxae. →



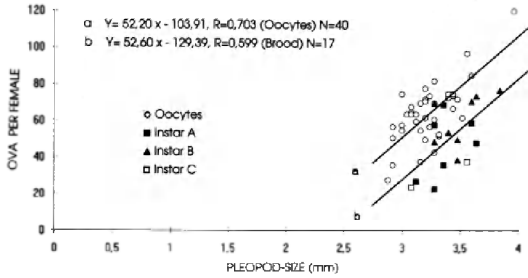


Fig. 2. Relationship between fecundity and pleopod size in females of *N. borealis*. Linear regression equations fitted to the data are given.

One specimen of instar D survived moulting into postmarsupial instar 2. This instar had six articles on the flagellum of the first antenna and lacked the seventh pereopods (second manca). The size of the first pleopod was 0.98 mm.

Broods developed synchronously within the same marsupium. No development of the marsupial eggs was observed in the laboratory when the temperature was 3.7-6.5° C. The observed duration of each of the marsupial instars is shown in Table 1. An increase in the average temperature of 1° C resulted in ten days longer development of instar B and C. Some of the eggs were infected by fungus at the highest temperatures. The duration of the three first marsupial instars was estimated to be 2.5 months at temperatures 9.0-9.5° C and 3.5 months at temperatures 9.5-11.0° C.

The number of oocytes found in the ovaries of *N. borealis* varied from 28 to 120 (Fig. 2). On average, the ovaries contained 64.9 oocytes (SD = 16.2, n = 40). The observed broods contained an average of 45.7 eggs in instar A, 60.5 embryos in instar B, and 31 larvae in instar C. The number of eggs, embryos and larvae found in the marsupia varied from 23 to 77, averaging 50.9 (SD = 17.8, n = 17). The

Table 1. The duration of marsupial instars of *N. borealis* in the laboratory

Marsupial instar	Time (days)	Temperature (° C)
A	25	9.0-9.5
B	25	9.0-9.5
B	35	9.5-11.0
C	25	9.0-9.5
C	35	9.5-11.0
D	70	11.0-10.0

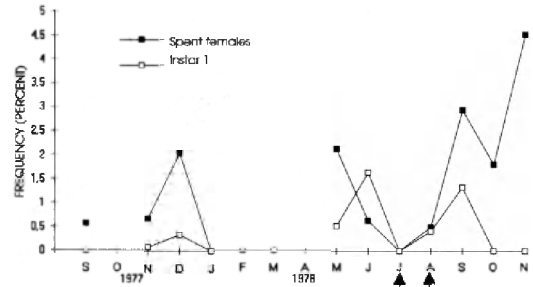


Fig. 3. Relative frequencies of instar 1 (pleopod 1 < 1.00 mm) of the total sample and relative frequency of spent females of the sexed females of *N. borealis* at Skogsvåg from September 1977 to November 1978. The arrows indicate months with significantly ($p < 0.05$) higher ratio of males to females than one.

linear regression of the numbers of oocytes in the ovary and the linear regression of the marsupial brood were significantly different from zero at the 1 % probability level.

A living brood was never found in the marsupium of females caught in the traps. Two dead eggs and two dead embryos were found on two occasions in the marsupium of females caught at Raknesvåg in 1995. Dead eggs and embryos in the marsupium were also observed twice in Skogsvåg. Oocytes at stage 2-3 were never observed in the ovaries of females with empty marsupia caught in the traps. The females with empty marsupia had probably released the brood (spent females) and were searching for food after brood release.

Peak catches were found in May-June 1978, September 1978, November 1978, and December 1977 at Skogsvåg of postmarsupial instar 1 and spent females (Fig. 3). The catches of postmarsupial instar 1 of *N. borealis* were lower at Skogsvåg than at Raknesvåg. Postmarsupial instar 1 was caught every month during the collections at Raknesvåg (Fig. 4). Postmarsupial instar 2 and 3 were caught throughout the year at both Raknesvåg and Skogsvåg. Spent females also occurred throughout the year at Raknesvåg. The maximum catches of postmarsupial instar 1 were made in January 1993, March and July 1994, and July 1995. The breeding activity of *N. borealis* thus seems to occur throughout the year while breeding intensity showed yearly variations without a clear pattern.

Sexual maturation and sex ratio

The appendix masculinum could be identified from instar 4 as a liberated and slender rod with a small process on the endopodite of the second pleopod. Sars (1899, pl. 29) has drawn this type of appendix in his description of *N. borealis*. The appendix rod later became moderately stout and bifurcated distally into two unequal processes tipped at their distal ends by a number of chitinous hooks or pads

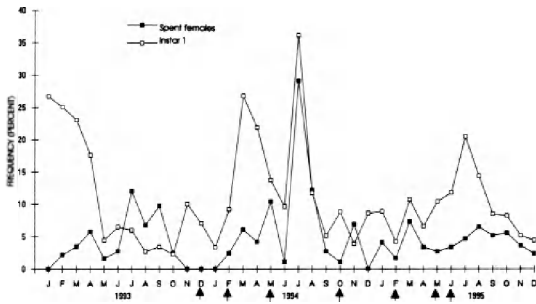


Fig. 4. Relative frequencies of instar 1 of the total sample and relative frequency of spent females of the sexed females of *N. borealis* at Raknesvåg from January 1993 to December 1995. The arrows indicate months with significantly ($p < 0.05$) higher ratio of males to females than one.

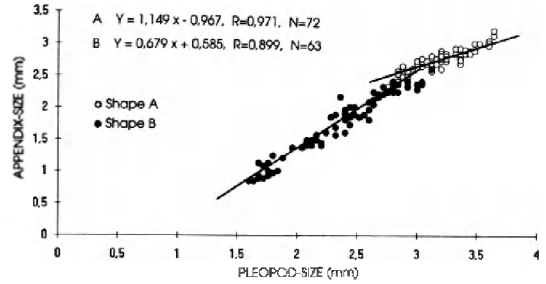


Fig. 5. Relative growth of appendix masculinum and pleopod in males of *N. borealis*. Linear regression equations fitted to the data are given.

(TATTERSALL 1905, pl. 9, fig. 8). The length of the process was half the width of the rod in young males (shape A). In large males the appendix masculinum became an edging along the rod and the length of the largest process was equal to the width of the rod (shape B). The relationship between the size of the appendix masculinum and the pleopod was described better by linear regression than by curvilinear regression (Fig. 5). The linear regressions of the relative growth for the two shapes were significantly different at the 1 % probability level. There was a change from shape A to shape B in the postmarsupial instar 6 and 7 in Skogsvåg and in instar 7 and 8 at Raknesvåg (Table 2). Shape B of the appendix was found on all of the males in the succeeding instars at both Skogsvåg and Raknesvåg. Most of the males had shape B in instar 7 both in Skogsvåg and Raknesvåg. Greyish-white semen was observed in the

vas deferens of some specimens of *N. borealis* from Raknesvåg with appendix masculinum of shape B. This was not observed in males with an appendix of shape A.

The females with marsupia from Skogsvåg were in instar 7-9, while marsupial females from Raknesvåg were in instar 8-10 (Table 3). Most of the marsupial females found in the laboratory were in instar 8, while instar 9 dominated in the field-material. The proportion of females with a marsupium (of all the females caught in the same instar) increased from instar 7 to instar 10 at Skogsvåg and from instar 8 to instar 10 at Raknesvåg. The proportion of marsupial females (of the females in the same instar) was higher at Raknesvåg than Skogsvåg for both instar 8 and instar 9.

The absence of incubating females in the traps, was expected to influence the sex ratio in favour of the males. The sex ratio of *N. borealis* was examined for instar 4-11

Table 2. Occurrence of shape A and shape B of appendix masculinum in males from Skogsvåg and Raknesvåg. The percentage of shape A and shape B are 100 % in each instar and given within the brackets.

Post-marsupial instar	Skogsvåg		Raknesvåg	
	Shape A	Shape B	Shape A	Shape B
	Sep-78		Sep-93,-94 & -95	
4	3 (100%)	0 (0%)	19 (100%)	0 (0%)
5	14 (100%)	0 (0%)	47 (100%)	0 (0%)
6	13 (92.9%)	1 (7.1%)	43 (100%)	0 (0%)
7	10 (21.7%)	36 (78.3%)	41 (42.3%)	56 (57.7%)
8	0 (0%)	30 (100%)	8 (8.8%)	83 (91.2%)
9	0 (0%)	13 (100%)	0 (0%)	80 (100%)
10	-	-	0 (0%)	5 (100%)
11	-	-	-	-
N	40	80	158	224

from Raknesvåg (Table 4). The ratios in instar 4 and 5 were not significantly different from unity. Equal number of males and females seems to be the norm among immature *N. borealis*. There was a slight, but significant tendency towards a larger proportion of males in instar 6 in the total sample from 1993-1995. A large part of the females in instar 7 and instar 8 were missing in the catches probably because they were incubating. The proportion of females in instar 9 was larger than in the two preceding instars, but still with a dominance of males. More females than males were caught in instar 10. Instar 11 was represented by three males. A female in instar 11 was also caught in January 1996. The increasing occurrence of females in instar 9 and 10 indicates a fairly equal mortality of males and females.

The total sex ratio (M:F) from Skogsvåg tended towards a dominance of females, while the opposite was true for Raknesvåg (Table 5). The relative number of

females caught at Raknesvåg, decreased from 1993 to 1995. The arrows in Figs 3 and 4 indicate months when the sex ratio was significantly ($p < 0.025$) higher than one. In Skogsvåg and Raknesvåg the low proportion of females coincided with low catches of instar 1.

DISCUSSION

Reproductive development

Natatolana borealis formed a fully developed marsupium in a biphasic moult. *Cirolana harfordi* (LOCKINGTON) also formed five pairs of fully developed oostegites in one moult (JOHNSON 1976). Mating was not observed for *N. borealis*. Copulation has rarely been observed among isopods, although the phenomenon of precopulative pairing is common (WHITE 1970). Sufficient depth of sediment seemed to

Table 3. Occurrence of marsupia in females from Skogsvåg and Raknesvåg. Females without marsupia from Skogsvåg were converted to instars from pleopod size: Instar 7: 2.76-3.16 mm, instar 8: 3.2-3.44 mm, instar 9: > 3.48 mm. Abbreviations: E.M., females with empty marsupium; Brood, females with brood in the marsupium

Post-marsupial instar	Skogsvåg					Raknesvåg			
	Laboratory			Field		Field			
	Nov-77 - May-79 E.M. (%)	Mar-79 Brood (%)	Sep-77 - Nov-78 E.M. (%)	Number of E.M. / total instar	E.M. as percentage of total instar	Jan-93 - Des-95 E.M. (%)	Number of E.M. / total instar	E.M. as percentage of total instar	
4	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	
6	0	0	0	0	0	0	0	0	
7	21.43	12.50	12.00	3/413	0.73	0	0	0	
8	53.57	68.75	36.00	9/347	2.59	10.48	11/197	5.58	
9	25.00	18.75	52.00	13/182	7.14	82.86	87/401	21.70	
10	-	-	-	-	-	6.67	7/10	70.00	
11	-	-	-	-	-	-	-	-	
N	28	16	25	25/942	942	105	105/608	608	

Table 4. Chi square analysis of sex ratio (M:F) of instar 4-11 from Raknesvåg. Abbreviations: n.s., not significant different from 1:1; M, male; F, female; N, total.

Instar	M:F	1993				1994				1995				Total			
		N	χ^2	p	M:F	N	χ^2	p	M:F	N	χ^2	p	M:F	N	χ^2	p	
4	0.79	269	3.58	n.s.	0.88	244	1.05	n.s.	1.00	232	0.00	n.s.	0.88	745	2.97	n.s.	
5	1.13	243	0.93	n.s.	0.95	242	0.15	n.s.	1.20	284	2.38	n.s.	1.10	769	1.59	n.s.	
6	1.29	188	3.07	n.s.	1.12	223	0.76	n.s.	1.26	242	3.24	n.s.	1.22	653	6.47	<0.025	
7	1.32	344	6.70	<0.01	1.49	371	14.37	<0.005	1.42	498	14.85	<0.005	1.41	1213	35.33	<<0.005	
8	1.13	321	1.13	n.s.	1.28	370	5.72	<0.025	1.53	407	17.75	<0.005	1.32	1098	20.49	<<0.005	
9	1.18	273	1.94	n.s.	1.26	378	5.12	<0.025	1.13	326	1.23	n.s.	1.20	977	7.75	<0.01	
10	0.63	13	0.77	n.s.	0.83	11	0.18	n.s.	0.50	9	1.11	n.s.	0.65	33	1.52	n.s.	
11	-	0	-	-	-	1	-	-	-	2	-	-	-	3	-	-	

be necessary in the aquarium to enable *N. borealis* to transfer oocytes from the ovary to the marsupium.

Moulting and mating on the sediment surface would increase the possibility of being eaten by predators. So the parturial moult and copulation presumably takes place in the burrow, which in *N. borealis* is U-shaped and penetrates to a depth of 7–10 cm into the sediment (TAYLOR & MOORE 1995).

Breeding females of *N. borealis* with marsupial instars A–C were never caught in baited traps. NIELSEN (1967) reports 12 specimens of *N. borealis* which carried embryos in the marsupium, and all these breeding females came from museum material. Information concerning the gear with which they were collected was available for only three of these females; all of which were caught by dredging. Female *N. borealis* with stage 3 oocytes were unable to have a full gut because of the voluminous oocytes in the ovaries. The brood in the marsupium was equally voluminous. Brooding females were physically able to feed, but obviously did not search for food under natural conditions. *Eurydice pulchra* LEACH and *E. affinis* HANSEN also accepted food during brooding, but fed only for short periods (JONES 1970). Marsupial female *N. borealis* were highly infected with the crustacean parasite *Cironiscus dahli*. These parasites in the marsupium might also influence the female behaviour in the same way as the brood itself, as is known from studies on *Rhizocephala* (NIELSEN 1967). *N. borealis* was able to double its dry weight in one meal. The increased volume of the gut pressed the hepatopancreatic sacs and ovaries towards the body walls (JOHANSEN 1980). The developing marsupial brood pressed the ventral body wall against the dorsum (NIELSEN 1967). The mechanical and physiological stimuli from the voluminous stage 3 oocytes or a full brood will thus impede breeding females from feeding.

Four marsupial instars were observed for *N. borealis*. The development of four marsupial instars of *E. pulchra* was described by JONES (1970). These instars resemble those observed in *N. borealis*. *Cirolana imposita* BARNARD also undergoes four marsupial instars (SHAFIR & FIELD 1980). DAVIS (1964) studied the hatching process of an ovoviviparous presumed *Cirolana* species. A single egg membrane was observed and the embryos were unable to survive outside the brood pouch. The eggs and the young were so firmly enclosed in the brood pouch of this species that they could only be removed by disruption of the body of the females. Since the brood pouch is formed by five pairs of overlapping oostegites which form an external pouch in species of *Cirolana*, it seems most likely that DAVIS (1964) examined an *Excirrolana* species (KLAPOW 1970).

The duration of development of oocytes in the ovary may deviate from three months at temperatures above 5–7°C. The temperatures observed in Skogsvåg at the collection site of *N. borealis* were 6.6–9.9°C during the col-

lection period (JOHANSEN 1980). Brood development was much slower at 6.3°C than at 11–16°C during the incubation of *Limnoria lignorum* (RATHKE) (SOMME 1940). Since the development of marsupial eggs of *N. borealis* was slow or non-existent at temperatures 3.7–6.5°C, these temperatures seem to be close to the lower limit for reproductive activity of this population. Instar A may have lasted longer than one month in broods developing at these low temperatures. Presence of fungus may have caused the increased duration of instar B and C at 9.5–11.0°C. Since marsupial instar D of *N. borealis* was able both to feed and swim, such individuals should be able to survive independently after the hardening of the exoskeleton. The last marsupial instar of *Idotea neglecta* G.O. SÆRS remained only a short time in the marsupium after the moult (KJENNERUD 1950). The fourth marsupial instar of *E. pulchra* lasted 8.5 days (of a total incubation of 57 days) and 5.5 days in *E. affinis* (of a total incubation of 37 days) (JONES 1970). The incubation period of *N. borealis* was estimated to be 3–4 months. The marsupial incubation of *C. hardfordi* lasted 3–4 months (JOHNSON 1976) and in *C. imposita* it was less than 4.5 months (SHAFIR & FIELD 1980). The total time for development of oocytes plus incubation in *N. borealis* was thus estimated to be 6–7 months. The female will not feed a large part of this period. Calculation of the energy budget and starvation experiments for *N. borealis* confirm that both oocyte-development and survival over the incubation period is possible after consumption of just one meal (JOHANSEN 1980). Reproduction of *N. borealis* may then largely depend on food availability. The availability of large food items might be unpredictable and not coupled with primary production, thereby resulting in a lack of seasonal pattern (SOONG & MOK 1994).

The trap used at Raknesvåg caught postmarsupial instar 1 of *N. borealis* efficiently throughout the year. The traps

Table 5. Chi square analysis of sex ratios (M:F) from Skogsvåg and Raknesvåg. Abbreviations: n.s., not significant different from 1:1; M, male; F, female; N, total.

Year	M:F	N	χ^2	p	Females (%)
Skogsvåg					
1977	0.98	940	0.07	n.s.	50.4
1978	0.87	2675	11.98	<0.005	53.4
Total	0.90	3615	9.67	<0.005	52.6
Raknesvåg					
1993	1.12	1651	5.47	<0.025	47.1
1994	1.18	1840	12.89	<0.005	45.8
1995	1.28	2000	29.77	<<0.005	43.9
Total	1.20	5491	44.26	<<0.005	45.5

used at Skogsvåg were probably less efficient and resulted in lower catches of instar I (JOHANSEN & BRATTEGARD 1996). The time of maximum reproduction varied from year to year at Raknesvåg and differed from the observed peaks at Skogsvåg. The breeding activity of *N. borealis* was continuous while the breeding intensity was variable. Similar observations were made for the deep-sea isopod *Bathynomus doederleini* ORTMANN from the depth of 300–500 m in waters of eastern Taiwan (SOONG & MOK 1994), while a seasonal pattern was suggested for the deep-sea isopod *Bathynomus giganteus* A. MILNE EDWARDS trapped at depths between 349 and 733 m off the Yucatan Peninsula (BRIONES-FOURZÁN & LOZANO-ALVAREZ 1991). The intertidal isopod *C. harfordi* breeds throughout most of the year, except for a brief lull in the fall (JOHNSON 1976). The population of the subtidal isopod *C. imposita* was characterized by continuous reproduction, with a three months lull in the summer when the temperatures reached a minimum (SHAFIR & FIELD 1980). ERVİK & al. (1982) measured 7.71° C in January 1982 and 7.31° C in May 1982 at 100 m in the Osterfjord. The temperature variation measured at the same depth and locality in the period October 1988 to August 1991 varied from 7.43° C to 8.46° C (JOHANNESSEN & al. 1990; JOHANNESSEN & al. 1991; TVEDTEN & al. 1994). The temperature in Osterfjord, at Raknesvåg at 90 m, was 8.1° C on the 1 June 1995 and increased slowly from 7.4° C to 7.6° C in the period 5–16 October 1995 (own obs.). The maximum temperatures were observed during the winter and minimum temperatures in the summer at both Skogsvåg and Raknesvåg. The recorded seasonal temperature variations were 1.03° C at Raknesvåg and 3.3° C at Skogsvåg.

Linear regressions between number of eggs and the pleopod length gave the best fit for *N. borealis*. The fecundity of cirrolanid isopods seems not to follow an exponential relationship (JOHNSON 1976). Linear relationships between brood size and body length of isopods were also found for *C. harfordi* (JOHNSON 1976), *C. imposita* (SHAFIR & FIELD 1980), *E. pulchra* and *E. affinis* (JONES 1970). The number of eggs in *N. borealis* declined from the ovary to marsupial instars A–C. Some oocytes may have remained in the ovary and been reabsorbed. The respiratory current from the pleopods and the lamina vibrans may have led to the loss of some eggs. Cannibalism was not possible in the marsupial instars A–C, since such young are unable to feed and, anyway, the development of the brood was synchronous. Dead eggs, embryos and larvae might have been eaten by the last marsupial instar. The mortality of *Janaira gracilis* MOREIRA & PIRES was estimated to be 39 % during the marsupial development (MOREIRA & PIRES 1977) while no reduction was experienced during the marsupial period in *I. neglecta* (KJENNERUD 1950).

Sexual maturation

The morphological change in shape of the appendix masculinum was associated with a change in relative growth of *N. borealis* and may be an indication of sexual maturation in the males. INAGAKI & BERREUR-BONNENFANT (1970) found a change in the allometric growth of the appendix masculinum and size of the pereon of *Ligia oceanica* (L.) at the male puberal moult. HARTNOLL (1982) writes that crustacean postlarval growth typically consists of one or more immature phases. The puberal moult is distinguished by clear morphological changes, and marks entry into the mature growth phase. The puberal moult need not coincide with the maturation of the gonads, but it almost invariably indicates entry into the instar in which sexual activity will commence.

The coincidence of sperm maturation and development of secondary sexual characteristics for *N. borealis* is supported by the presence of semen in the vas deferens of males with copulatory stylet of shape B. It seems possible that only one androgenic hormone exists, which controls both the primary and secondary male characteristics in malacostracans (CHARNIAUX-COTTON & PAYEN 1985).

The small genital papillae of adult males of *N. borealis* will probably not reach the gonopores of the females. The bifurcated appendix masculinum of shape B with the small hooks of *N. borealis* may have a functional task during copulation. In most isopods, the endopodites of the second pair of pleopods are differentiated as copulatory stylets that function either by accretion or intromission (CHARNIAUX-COTTON & PAYEN 1985). The appendix masculinum was involved in the copulation of the didoteid isopod *Glyptonotus antarcticus* (EIGHTS) and a groove facilitated the spermato-phore transfer (WHITE 1970). In *Asellus aquaticus* (L.) the fifth thoracic segment of the female was positioned immediately below the second pleopod of the male during copulation. Such an arrangement would bring the second abdominal appendages into the immediate neighbourhood of the female reproductive openings. Spermatozoa were found in the receptacles within the gonopore openings of the fifth thoracic segment of female *A. aquaticus*. After being released from the male the anterior ecdysis was completed and eggs transferred (UNWIN 1920).

Shape B of the appendix masculinum is retained for life, so that mature male *N. borealis* may thus fertilize more than one female. *Mesidotaea entomon* (L.) also retained this mature appearance (HAAHTELA 1978).

Assuming that shape B of the male appendix accompanies with sexual maturity, sexual maturation was achieved over two instars. Some of the males became mature from instar 6 at Skogsvåg and from instar 7 at Raknesvåg. Most of the males matured in the following instar. In succeeding instars all of the males were mature. Taking the development of oocytes in the ovary into account, the females

must have been sexually mature in the instar prior to the marsupial instar. Females therefore must have attained sexual maturity in instar 6 at Skogsvåg and instar 7 at Raknesvåg. Sexual maturity of *N. borealis* was thus attained at an age of 12-18 months at Skogsvåg (JOHANSEN 1980). Sexual maturity of *N. borealis* from Loch Fyne, Scotland was estimated to be achieved after 19 months (WONG & MOORE 1996).

Sex ratio

Instar 7, 8, and 9 were biased towards the males at Raknesvåg. At least some of the females in instar 7 and 8 were sexually mature. Incubating females were not caught in traps. Some of the females were presumably incubating at the time of sampling, and this will have influenced the sex ratio. The sex ratio of instar 6 in the total catch from Raknesvåg was slightly, but significantly biased toward the males, since the ratios in immature instars were close to one. This indicates that some females may also have become sexual mature in this instar.

Male *N. borealis* dominated at Raknesvåg in 1993-1995 (45.5 % females of a total of 5 491 males and females trapped). The sex ratios of *N. borealis* from trap collections in Loch Fyne, Scotland were mostly significantly male-dominated (WONG & MOORE 1996). NIELSEN (1967) found 47.7 % females of a total of 3 521 *N. borealis* collected from 30 localities.

Females, however, dominated in the traps at Skogsvåg (52.6 % of a total of 3 615). NIELSEN & STRÖMBERG (1965) caught 51 % females of a total of 3 713 from 90 m at the same locality. The difference between Skogsvåg and Raknesvåg may be a result of different environmental factors. The small size-differences in the funnel entrances of the traps would probably not favour a particular sex. Differential migration, longevity, growth rate, or mortality, however, might also alter sex ratios (WENNER 1972).

Number of broods

Moulting of the marsupium of *N. borealis* was not observed, but several indications point to an intergenerative instar after the incubation. The spent females of *N. borealis* in the traps had not developed oocytes in the ovary. Since female *N. borealis* must have starved a long time during the brooding period, they would probably eat, moult and then develop the oocytes in the next instar. Scars could sometimes be seen, resembling the edge of the brood pouch, on the caudal ventral integument of some adult females without a marsupium. This was interpreted as possible traces of an earlier marsupial instar. The need for a resting period after starvation during incubation is obvious. Female *C. imposita* retained empty marsupia for 35-40 days until the next moult when the oostegites were shed (SHAFIR

& FIELD 1980). Shedding of the marsupium and an intergenerative phase of breeding has also been observed in *M. entomon*, *A. aquaticus* (HAAHTELA 1978), *E. pulchra*, and *E. affinis* (JONES 1970). These mature females might then be erroneously classified as immatures.

High mortality of females after reproduction is usual for isopods (MOREIRA & PIRES 1977). Spent female *N. borealis* were rare in present catches. This may be caused by both high mortality after reproduction and by reproduction throughout the year. The relatively high occurrence of females in instar 9-11 indicates equal mortality of both sexes. If mortality of the females was high due to reproduction, it must have been equally high in males. Since the males do not incubate, they might be exposed to more predation.

Nine postmarsupial instars of *N. borealis* were observed from Skogsvåg and eleven postmarsupial instars from Raknesvåg. Female *N. borealis* carrying a brood at instar 7 at Skogsvåg and at instar 8 at Raknesvåg can thus have two broods. A large proportion of females with a marsupium were in instar 8 from Skogsvåg and instar 9 from Raknesvåg. Most likely those females would have not more than one brood. WONG & MOORE (1996) suggested semelparity for *N. borealis* from Loch Fyne. The females of *C. harfordi* could produce three or more broods in the laboratory, but the survival of the females beyond a second brood under natural conditions seemed poor (JOHNSON 1976). *C. imposita* also probably did not survive beyond the release of a second brood in nature (SHAFIR & FIELD 1980). *N. borealis* seems to be a K-strategist. K-selection implies delayed maturity, small number of young, iteroparity, parental care, and a small reproductive effort during the lifetime of an individual (SASTRY 1983).

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