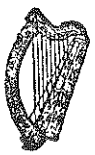

IRISH FISHERIES INVESTIGATIONS

SERIES B (Marine)

No. 16 (1975)

AN ROINN TALMHAIOCHTA AGUS IASCAIGH
(Department of Agriculture and Fisheries)

FO-ROINN IASCAIGH (Fisheries Division)

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CAPTIVE REARING OF LARVAE OF THE DUBLIN BAY PRAWN *NEPHROPS NORVEGICUS* (L)

Captive rearing of larvae of the Dublin Bay prawn *Nephrops norvegicus* (L.)

by J. P. Hillis.

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(Received 28th November, 1974).

Abstract

Wild caught *Nephrops* larvae were maintained in aquaria for as long as possible, in 1969, 1970 and 1971 together with a few which were hatched in the laboratory in 1971. Experiments were conducted at temperature ranges of 16°C to 22°C and 11°C to 13°C; the latter range is close to the ambient temperature of larvae in the sea. Direct observations were obtained of the duration of all stages from first larval to third post larval (six successive stages in all), though percentage survival rates were rather low in some groups.

Introduction

The three larval stages of *Nephrops norvegicus* were first described by Sars (1884, 1889) and have since been described by a number of authors, of whom Santucci (1926, 1927) was one of the earliest to give a very detailed account. Their occurrence in nature has been described by Jorgensen (1923, 1924, 1925), Karlovac (1953), Andersen (1962), O'Riordan (1964) and Hillis (1968, 1972c, 1974).

The three stages between hatching and the metamorphic moult are easily identified by the abdominal pleopods which are absent, small and large in the first, second and third stages respectively. In addition, the supraorbital spines are first seen in the second stage and the uropods in the third. Thus, by the development classification of Williamson (1969 and pers. comm.), the first stage is a zoea (pleopods absent), as is the second if its small non-setose abdominal pleopods be defined as rudimentary. The third stage employs both setose exopodites of the pereipods and setose abdominal pleopods, which are zoeal and megalopal characteristics respectively, as sources of propulsion and may hence be considered as transitional between a zoea and a megalopa. A recent suggestion that the species hatches into a pre-zoeal stage of several minutes duration which then moults to give the first zoea (Farmer, 1974) is considered erroneous (Hillis, in prep.). To Andersen (*op. cit.*) the three stages were "zoea", "first mysis" and "second mysis" respectively. In this paper, they are simply referred to as stages 1, 2 and 3. The abbreviations PL1, PL2 etc. are used to designate the postlarval (or early juvenile) stages, resembling miniature adults in most respects, which succeed the larval phase.

Rearing of larvae in the laboratory was undertaken to obtain data on stage duration, as well as to make observations on stage 3 larvae, described as rare by Andersen (*op. cit.*) and O'Riordan (*op. cit.*), and on early post-larvae, rarely observed prior to the present investigations (Hillis 1972a, 1972c).

The rearing of decapod larvae has a rather long history, having first been undertaken to identify unknown larvae and to classify types of development, and also—especially in the case of the lobsters, *Homarus* spp.—with a view to developing farming methods. Important early taxonomically orientated work was that of Lebour in the twenties (see Russell, 1972) since when much work has been done, mainly in eastern Asia, America and Europe. In contrast to *Homarus* spp. *Nephrops norvegicus* larvae have not been widely kept in captivity though Foxon (1934) made observations on the method of swimming. Preliminary results of the present work, including hatching of attached eggs in the laboratory, have already been noted (Hillis 1970, 1971, 1972b) and eggs detached from ovigerous females have recently been hatched in Portugal (Figueiredo, 1971, Figueiredo and Villela, 1972). Regarding technique in crustacean rearing, Provenzano (1967) stressed the importance of larval *Artemia salina* as food. The recent general review of rearing methods by Rice and Williamson (1970) however, only became available towards the end of the present work.

METHOD

Collection

Larvae collected at sea (mainly during larval surveys) were brought to the laboratory, examined, separated into stages and put into permanent accommodation as quickly as possible. In 1970 and 1971, they were separated by stage on capture at sea and then re-examined in the laboratory. From June 1970, vacuum flasks were used for conveying stock from research vessel to laboratory to reduce mortality en route during warm weather.

Where larvae were collected on several successive days during a cruise, they were separated by day of capture as well as by stage. Naturally, the longer the interval between capture and laboratory examination the higher was the proportion of larvae to have moulted or died therein; in some cases where the only catches yielding reasonable numbers occurred early in a cruise, this interval might be several days.

In addition to the collection of larvae at sea, small scale hatching of eggs on ovigerous females in the laboratory was undertaken in 1971 (Hillis 1972b). Data of laboratory hatched larvae which underwent complete stages in the laboratory are compared here with similar data for wild larvae.

Maintenance

Larvae were generally kept singly in cages made of PVC and nylon gauze standing with their sides above water-level in glass or fibre glass aquaria filtered by standard proprietary filters using diffusion stones, activated charcoal and glass wool. Numbers of larvae of the same stage which had previously moulted in captivity on the same date or not at all were in some instances kept together.

In some cases, cages were dispensed with and the larvae kept at large in the aquaria. In 1969 a "plankton-kreisel" built broadly to the specifications of Greve (1968), was used but as this was found less simple to maintain than other accommodation and had no special advantage in this work, its use was discontinued after a short period.

A small scale experiment using 250 ml beakers without aeration but with total replacement of water each day, undertaken on 21st May, 1969, gave the following survival rates (shown as fraction of initial stock surviving 24 hours with percentages in brackets):—

Stage	1	2	3	Overall
No aeration	1/6 (17%)	5/10 (50%)	3/5 (60%)	9/21 (43%)
Aeration supplied	4/5 (80%)	5/5 (100%)	3/3 (100%)	12/13 (92%)

These results pointed strongly to the need for aeration, which was supplied in all accommodation thereafter.

Larvae were fed larval *Artemia salina* daily, supplemented in stage 3 and replaced in the post-larval phase by pieces of mussel *Mytilus edulis* gonad and small squid *Alloteuthis* sp. flesh. The *Artemia* were obtained in egg form, and hatching and survival rates were very much better in 1969 than in 1970 or 1971.

Daily readings of temperature were made between 9.30 and 11.30 hrs. The maxima and minima of these readings over ten day periods are given in Table 1. The diurnal temperature range recorded over six months April-September 1971 had a value of from 0.5°C to 2.8°C, mean 1.3°C, the values recorded at the above mentioned morning readings generally falling above the minimum by approximately one third of the range.

At the beginning of July, 1971, a cooling bath became available and thereafter all larvae were reared in tanks standing in this. It consisted of a galvanised steel trough of approixmate dimensions 2.4 × 0.5 × 0.5 m, through which fresh water was circulated, the water going through metal coils in a thermostatically controlled cooler immediately prior to entering the bath. The *Nephrops* tanks stood on blocks in this tank which was filled with fresh water to approximately the same level as the sea water in the tanks. The temperature was maintained at about 11°-13°C.

Daily examination of specimens for moults, injuries or death was by X10 binocular microscope, larvae being handled with a small flat gauze lifting net or by optician's forceps. To minimise disturbance, larvae not due to moult, because they had just done so, were normally not examined.

RESULTS

The numbers of larvae commencing each experiment and achieving one or two moults are given in Table 2. The percentage ratios given in Table 3 compare success-rates between stages using pooled data of all years because of the limited numbers of observations, and between years similarly using pooled data of all stages. These indicate that larvae caught at stage 3 survived markedly more successfully than those caught at stages 1 or 2, and that while survival from introduction to first captive moult was poorer in 1969 than in subsequent years, survival to the second captive moult was very much better. The better rate of long-term survival in 1969 than in 1970 or 1971 is probably due to the better hatching qualities of the *Artemia salina* used in that year.

Whilst survivors to the second captive moult were not numerous, they did provide observations on the duration of all stages from the first larval to the third post-larval.

The total numbers of observations of stage duration were as follows:—

Stage :—	1	2	3	PL1	PL2	PL3
No. of observations :—	2	12	5	13	6	3

The individual durations of these are set out in Table 4. They include six imprecisely-known values, provided by specimens which were in stage 1 on capture at sea on 19th/20th May, 1970, but had moulted to stage 2 prior to re-examination on arrival at the laboratory on 22nd May.

A noticeable phenomenon was the occurrence of cases of partial moulting, where a specimen commenced a moult but could not complete it, and was left with the old exoskelton still firmly attached in a partly discarded position. Where such moults separated stages 1 and 2 or 2 and 3, the cephalothorax was usually seen to be raised high posteriorly, but still fixed anteriorly, causing the rostrum to point markedly downwards. For the metamorphic (3/PL1) and post larval moults, non-completion was less widespread and more variable in form, completion of moult except for failure to withdraw pereipods from the old shell being sometimes noted. Death usually followed an incomplete moult within a day in larvae, but with post-larvae survival was sometimes longer, a specimen surviving in moult PL2/PL3 for nine days in July 1969, and one in moult PL1/PL2 for six days in August 1971, while two specimens which were artificially assisted in completing their moults survived for 15 days in stage PL2 during July-August 1970 and 13 days in PL3 in September 1971. There was however no record of a larva which underwent an incomplete moult surviving to moult again.

Table 5 gives the means of the stage-duration values shown in Table 4. Each stage is classified by year to show the difference between 1971, when the cooling apparatus was in use, and earlier years, when the ambient temperature was dictated by room temperature. In addition, duration data for stages ending with incomplete moults and for those ending with normal moults are separated.

Whilst the low numbers necessitate caution in interpretation, it would appear that the most important values are those of 10, 13, 16, and 28 days for normally terminated stages 1, 2, 3, and PL1 respectively obtained in 1971, at a temperature in the region of 11°C-13°C (see Table 1), which is probably not much different from those surrounding larvae in nature (Hillis, 1974). While these values are based on very few specimens (notably the longer surviving one of the two laboratory-hatched specimens), and while the stage durations of specimens kept at room temperature in 1969 and 1970 (16°C-20°C, see Table 1) were greater by a factor of 1.6 approximately, nevertheless the ratios of the durations for stages 3:2 and PL1:3 were very similar for the two sets of data, making this factor very consistent for stages 2, 3 and PL1 at 1.6 in each case (though very different for PL2 and PL3) and this triplication of approximately the same factor suggests that it may have some reliability as a conversion factor for duration (T°cooler)/duration (T°room) values, thus tending to reinforce the limited 1971 data.

A comparison of durations for stages terminating in incomplete moults with those ending in normal moults shows a consistantly higher value for stages where the terminating moult is incomplete, by factors in the range 1.1 to 1.3. This suggests that postponement and incompleteness of moulting may be two effects of the same cause, probably some form of general debility involving reduced production of a moult-inducing hormone. Because of this estimates of the duration of a stage based on the maximum time spent therein by captive specimens, where dates of the moults entering that stage cannot be obtained, are not reliable.

This phenomenon can be further illustrated by reference to one large group of larvae, namely those in stage 1 when introduced to the laboratory on 22nd May 1970. Table 6 shows the incidence of different types of termination (normal moult, incomplete moult, and death unmoulted) both of the stage 1 period by days

subsequent to introduction to the laboratory and of the stage 2 period by days subsequent to the stage 1 to 2 moult where this had been successful. (In the latter case, certain low values can only be represented as < 3, < 4 and < 5 days), due to the practice, in the interests of minimising disturbance, of non-examination of stock not due to moult soon. With the stage 1 larvae, maximal captive durations in the stage are of interest (not in the mean durations, which are strongly influenced by many deaths in days one to three, following transit to the laboratory). With one exception, which performed a normal moult by day twelve, the maximum durations were six days for normal moults, eight days for abnormal moults and eleven days for most deaths unmoulted, with however one exceptional specimen dying unmoulted by day twenty-one. The same effect of extended *maximal* durations can be seen in the case of the stage 2 specimens after one captive moult.

At other times causes of death included cannibalism. Larvae were seen to attack others on a number of occasions and a number of severely mutilated specimens occurred with eyes and pereipods the parts most frequently attacked. Very dark matter observed in the gut in individuals in groups where cannibalism was rife was believed to be ingested eye pigment.

In addition to cannibalism, malnutrition and possibly bacterial infection appear to have taken their toll. Malnutrition would tend to promote cannibalism and this may have been the case in 1970, when numbers were large, and the *Artemia* hatched much less readily than the previous year. Dead specimens frequently harboured populations of ciliates, and this feature and the replacement which normally occurs after death of the body transparency normal in very young *Nephrops* by an opaque appearance were sometimes noted in ailing specimens where they were a reliable indicator of impending death. Rice and Williamson (1970) have noted ciliates associated with moribund crustacea and consider that these may appear in response to a food supply of bacteria.

It is not possible to be certain from the data whether temperature played a major role in determining mortality in these experiments, though this has been found to be the case elsewhere (Figueiredo, 1971).

DISCUSSION

The experiment as a whole showed that *Nephrops* could be maintained in captivity throughout the larval phase and for a long period into the post-larval/juvenile phase though long survival in captivity was only achieved by a limited proportion of the total number introduced.

A valuable aspect of the experiment is that while the normal duration of each stage in the wild population in the Irish Sea remains unknown (they are considered probably to be similar to those of the long surviving laboratory-hatched specimen in 1971), laboratory findings of relative durations of the stages (Table 6) are of value in assisting interpretation of certain findings of research cruises. For example, the fact that stage 2 larvae attained a maximum of 40-42% of the total larval catch in all three seasons surveyed (Hillis, 1974) might be assumed to indicate that stage 2 occupied somewhere in the region of 40% of the total duration of the larval phase were it not for the laobratory evidence that it occupies between 30 and 35%, suggesting that some factor is introducing bias in larval sampling, possibly under-representations of stage 3 in catches due to some of them having already descended to the sea-bed.

It appears that rearing of this species commercially could be difficult owing to the high incidence of cannibalism, necessitating individual accommodation.

Figueiredo (1971) found hatching success to be inversely correlated to temperature within the range 11°-18°C, whilst success in completion of larval stages was affected in the same way, but to a much lesser degree. The inverse correlation of stage duration with temperature was also noted but Figueiredo does not give exact measurements of stage durations for larvae or post-larvae. Her longest surviving specimen died in the third post-larval stage, about four months after hatching, surviving for a period not far short of the longest survivor in the present work which died in the fourth post-larval stage, 164 days after hatching.

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SUMMARY

1. Methods by which *Nephrops norvegicus* larvae were maintained alive in the laboratory are described.
2. Observations of duration of every stage from first larval to third post-larval are given. In one case, a specimen hatched in the laboratory underwent all six stages.
3. The significance of different results obtained at different temperatures is examined.
4. An association between postponement of moulting and uncompleted moults is described.
5. Various possible causes of death in captivity are discussed.

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Table 1. Aquarium temperature (T°C) at daily readings summarised in ranges over 10 days periods (approximate in last period of month), 1969-1972.

Period	T°C 1969	T°C 1970	T°C 1971	Period 1971—1972	T°C
May 1	—	16.7-19.5	—	Nov. 1	9.6-11.6
2	—	16.0-18.9	—	2	10.7-12.1
3	16-19	15.6-17.4	—	3	11.6-12.1
June 1	17-19	17.1-22.3	—	Dec. 1	12.0-12.6
2	16.3-20.0	19.1-23.6	—	2	11.3-12.7
3	16.4-19.0	18.0-19.1	15.1-17.6	3	11.6-12.7
July 1	15.7-19.7	16.8-21.1	12.1-20.5*	January 1	10.7-13.0
2	18.4-22.0	17.8-19.3	11.7-12.4	2	11.6-12.6
3	20.9-22.0	17.2-19.6	11.6-12.5	3	10.5-12.5
August 1	—	19.9-21.9	11.6-11.9	Feb. 1	10.2-12.1
2	—	16.8-20.8	11.5-12.4	—	—
3	—	17.2-20.2	11.2-11.7	—	—
Sept. 1	—	17.5-19.1	11.5-12.6	—	—
2	—	14.4-17.8	11.4-12.4	—	—
3	—	17.0-21.4	12.0-12.5	—	—
October 1	—	13.3-16.2	12.3-12.6	—	—
2	—	—	11.9-12.3	—	—
3	—	—	12.0-12.4	—	—

* cooling bath started operating, 5-7-71.

Table 2. Numbers of larvae at commencement of experiments and undergoing first and second captive moults.

Stage at Capture	Year	Experiment starting date	Number of days since capture	NUMBERS OF LARVAE: At start of experiment A	Total 1st moults B	1st moult normal C	Total 2nd moults D	2nd moult normal E
1	1969	20.5	(1)	15	3	3	—	—
	„	26.6	(1)	3	—	—	—	—
	1970	22.5	(3-2)	138	83	69	11	6
	1971	25.6	(1)	12	9	7	—	—
	„	29.6	(1-0)	2	1	—	—	—
	Total			170	96	79	11	6
2	1969	20.5	(1)	15	5	5	2	1
	„	12.6	(1)	2	—	—	—	—
	„	26.6	(1)	1	1	1	1	1
	1970	22.5	(3-2)	23	11	3	1	1
	„	27.6	(1)	15	10	8	—	—
	1971	25.6	(1)	4	3	3	—	—
	„	29.6	(1-0)	2	1	1	—	—
	Total			62	31	21	4	3
3	1969	20.5	(1)	3	1	1	1	1
	„	12.6	(1)	4	2	2	2	2
	„	26.6	(1)	1	—	—	—	—
	1970	27.6	(4-1)	44	30	26	6	3
	1971	25.6	(1)	12	6	5	2	2
	„	29.6	(1-0)	2	1	1	1	—
	Total			66	40	35	12	8
Annual Overall totals	1969			44	12	12	6	5
	1970			220	134	106	18	10
	1971*			34	21	17	3	2
Laboratory-Hatched, August-September, 1971				32	2	2	1	1
GRAND TOTAL				330	169	137	29	18

* Excluding laboratory hatched data.

Table 3. Summarised percentage survival ratios (see columns A-E in Table 2).

Ratio (%)	Stage at capture (all years) 1	2	3	Year (all stages) 1969	1970	1971*
C/A	43	34	53	27	48	50
E/A	4	5	12	11	4	6
D/C	14	19	34	50	17	18
E/C	8	14	23	42	9	12

* Excluding laboratory hatched data.

Table 4. Durations of stages in days. Part stages (observed period commencing with capture or ending with death) are shown in brackets.

* = last complete stage terminating in incomplete moult.

** = last complete stage terminating in moult completed with artificial aid.

A. LARVAL STAGES

Date of capture (or hatching, H)	Duration (days) of stages 1	2	3	PL1	Date of death	Total no. of larval stages	Total duration of captivity (days)
1969: 19 May	—	(5)	11*	(0)	4 June	1*	16
„	—	(6)	8	(15)	17 June	1	29
25 May	—	(2)	10	(17)	24 July	1	29
1970: 19-20 May	(0-3)	ca7	(11)	—	8 June	1	19-20
„	(0-3)	ca7	(3)	—	31 May	1	11-12
„	(0-3)	ca7	(2)	—	30 June	1	10-11
„	(0-3)	ca8	(14)	—	12 June	1	23-24
„	(0-3)	ca9*	(2)	—	1 June	1*	12-13
„	(0-3)	ca11*	(0)	—	1 June	1*	12-13
„	(5-6)	9	(6)	—	9 June	1	20-21
„	(5-6)	10*	(0)	—	4 June	1*	15-16
„	(5-6)	10*	(1)	—	5 June	1*	16-17
„	(5-6)	11*	(0)	—	5 June	1*	16-17
„	(7-8)	7	(9)	—	12 June	1	23-24
„	—	(5-6)	12	(7)	13 June	1	24-25
1971: H 31 Aug.	10	2	—	—	12 Sept.	1	12
„	10	13	16	(continued in Part B)		3	39 (larval)

B. POST-LARVAL STAGES

Date of Capture	3	PL1	PL2	PL3	PL4	Date of death	Total no. of post-larval stages	Total duration of captivity (days)
1969: 19 May	(8)	13	(7)	—	—	17 June	1	29
11 June	(6)	20	(8)	—	—	14 June	1	33
11 June	(1)	14	18*	(9 @ PL2/PL3)	—	23 July	2*	42
1970: 24 June	(7)	21*	(0)	—	—	22 July	1*	28
24 June	(5)	19*	(0)	—	—	18 July	1*	24
26 June	(8)	19	(15)	—	—	5 Aug.	1	20
26 June	(5)	19**	(15)	—	—	4 Aug.	1**	39
26 June	(7)	15	18	19	(40)	3 Oct.	3	99
26 June	(6)	19	24*	(1)	—	17 Aug.	2*	52
1971: 25 June	(6)	26	43	32	(34)	13 Nov.	3	141
24 June	(6)	25	43**	(13)	—	21 Sept.	2**	87
29 June	(2)	33*	(6 @ PL1/PL2)	—	—	9 Oct.	1*	41
(H 31 Aug. continued from Part A)		28	42	45	(10)	11 Feb. '72	3 (post-larval) 6 (total)	125 (post-larval) 164 (total)

Table 5. Mean stage length in days ($\overline{D}$), its standard deviation (SD) and numbers (N) of complete stages undergone ending successfully (A) and unsuccessfully (B), by years.

Stage	Parameter	1969 A	1969 B	1970 A	1970 B	1969 & 1970 Combined A	1969 & 1970 Combined B	1971 A	1971 B	RATIOS B/A (1969-70)	RATIOS 1971/1969-70 (A)
1	$\overline{D}$	—	—	—	—	—	—	10.0	—	—	—
	SD	—	—	—	—	—	—	0	—		
	N	—	—	—	—	—	—	2	—		
2	$\overline{D}$	—	—	8.0*	10.3*	8.0	10.3	13	—	1.3	1.6
	SD	—	—	1.41	0.58	1.41	0.58	—	—		
	N	—	—	2	3	2	3	1	—		
3	$\overline{D}$	9.0	11	12	—	10.0	11	16	—	1.1	1.6
	SD	1.41	—	—	—	2.00	—	—	—		
	N	2	1	1	—	3	1	1	—		
PL1	$\overline{D}$	15.6	—	17.6	19.6	16.6	19.6	26.3	33	1.2	1.6
	SD	3.79	—	2.31	1.15	3.01	1.15	1.53	—		
	N	3	—	3	3	6	3	3	1		
PL2	$\overline{D}$	—	18	18	24	18	21.0	42.5	44	1.2	2.4
	SD	—	—	—	—	—	4.24	0.71	—		
	N	—	1	1	1	1	2	2	1		
PL3	$\overline{D}$	—	—	19	—	19	—	38.5	—	—	2.0
	SD	—	—	—	—	—	—	9.19	—		
	N	—	—	1	—	1	—	2	—		

* Periods of imprecisely known duration excluded.

Table 6. Occurrence with time of different types of termination of stage in larvae introduced to the laboratory at stage 1 on 22nd May 1970.

Day Number	A. Stage 1; from start of experiment till first captive moult or death.				B. Stage 2; from first captive moult till second captive moult or death.			
	Normal moult	Abnormal (incomplete) moult	Death	Total	Normal moult	Abnormal (incomplete) moult	Death	Total
1	6	4	18	28	—	—	3	3
2	11	5	15	31	—	—	8	8
3	12	1	1	14	—	—	7 (+ 1 @<3)	7(+ 1)
4	7	—	1	8	—	—	1 (+ 5 @<4)	1(+ 5)
5	4	—	3	7	—	—	(6 @<5)	(6)
6	1	—	—	1	—	—	—	—
7	—	1	—	1	1	—	1	2
8	—	2	3	5	—	—	—	—
9	—	—	1	1	1	—	1	2
10	—	—	1	1	—	2	1	3
11	—	—	2	2	—	1	—	1
12	1	—	—	1	—	—	—	—
13	—	—	—	—	—	—	1	1
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	1	1
20	—	—	—	—	—	—	1	1
21	—	—	1	1	—	—	—	—
Total	42	13	46	101	2	3	37	42

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