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- [ ] NEW PROPOSAL
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- [x] FINAL REPORT
- [ ] PROGRESS REPORT

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: Nursery and container culture of blacklip abalone

ORGANISATION: Dept. of Sea Fisheries, Tasmania

PERSON(S) RESPONSIBLE: Mr MA Wilson

FUNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1984/85		$31,661
1985/86		$15,595

RELATED APPLICATIONS:

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inspect his catch as it was boarded. This approach was also used during this program, but with limited success; with only ten abalone from the several thousand inspected producing gametes.

4. A professional abalone diver was employed to harvest an area (Port Arthur) that had recently contained ripe abalone. The exhorbitant cost of employing a professional diver, and difficulties in administering licence conditions precluded the continuing use of this approach. Although mature abalone had been collected by Departmental divers in the area the previous week, none were obtained on this occasion.

5. Abalone processors were visited on several occasions and the abalone inspected during processing. In this manner abalone harvested from the whole of the coast-line could be sampled rapidly to determine whether a more detailed effort was warranted in a specific area. No abalone were obtained for spawning from processors as they were usually in poor (for our purpose) holding conditions, and in several factories, the gonad could only be inspected after the abalone had been shucked.

6. Collection and inspection of abalone by divers employed by this Department resulted in the procurement of the majority of the brood stock used in spawning attempts. Although the bulk of this effort was directed at the South-East coast from the Acteon Islands to Bicheno, much of the State's coast-line was represented, with areas such as Flinders Island, Rocky Cape and Temma being sampled on occasion. Sampling of these more remote areas was unfortunately restricted by personnel and resource availability. Approximately 20,000 abalone were individually inspected in this approach, and resulted in less than 50 abalone that produced gametes in hatchery.

Investigations of the gametogenesis of Haliotis ruber (Harrison and Grant, 1970), and previous successful culture work performed by this Department imply that black-lip abalone may be capable of effecting a number of spawnings over a protracted period.

The experience of this program was that very localised pockets of ripe abalone were found. This has also been found with other species (Mottet, 1978a; Young and DeMartini, 1970). When one of these pockets was located, usually 10% of the abalone inspected would be selected on a macroscopic gonad bulk criteria, and 20-50% of these would produce gametes (viable or not) in the hatchery. Each pocket supplied only one batch of stock, as when re-visited to obtain further stocks, sometimes the next day, and always within two weeks no further ripe abalone were found in that or immediately adjacent areas.

Dr Len Tong, who is conducting similar work in New Zealand, has discontinued using the macroscopic gonad bulk index when selecting H. iris and H. Virginea for spawning. He claims reliable spawnings using stock which has been conditioned in the hatchery for up to six months irrespective of gonad bulk. He has been unable to spawn H. australis for as yet unknown reasons. (Pers. Comm., 1985).

A condition previously unencountered in H. ruber, where the female gonad was visibly distended, and the animal scored a three rating on the macroscopic gonad bulk index (fully ripe, competent to be spawned), yet on histological inspection there was no gonad material present; was observed. This condition has also been recorded in Japanese species (Thomson, 1983).

As this past year has been one of unseasonally warm water temperatures, with minimum recorded water temperatures on the East coast approximately three degrees Celcius higher than usual, and abnormal rain (wettest December for 70 years, January 64 mm above average), it is possible that the spawning strategy exhibited during the course of this program is not the usual one, and that in future, stocks of ripe abalone would be readily available as previously.

Conditioning

The unavailability of good brood stock during the contracted hatchery time at Bicheno in May 1985 precipitated an attempt to utilise the remaining hatchery time to investigate conditioning techniques applicable to H. ruber. It soon became apparent that an effective conditioning trial would require more extensive and sophisticated resources than the hatchery and Department could at that time provide. This aspect has been recommenced in conjunction with Tasmanian Shellfish Company facilities.

Spawnings

Several techniques were used in attempts to spawn the abalone that were collected. The Ultraviolet light irradation technique developed by the Japanese (Kikuchi and Uki, 1974a), and used previously by this Department, and the hydrogen peroxide technique (Morse et al, 1977) favoured by the Americans formed the basis for our approach. Serotonin, a neurotransmitter known to induce spawning in bivalve molluscs (Gibbons and Castagna, 1984) was also used to a lesser extent.

Early in this work, it was felt that the UV sterilizer used previously was not functioning as it should, and this therefore was replaced at a cost of $2,000.

Twenty spawning attempts were made during the past twelve months using brood stock of varying competency. The best stock, and the only stock to result in the production of larvae, were collected on

one day at Port Arthur. Second and third visits to the same spot the next day, and one week later, were fruitless. Of the 680,000 eggs which were slowly trickled out by three females, less than 1% fertilised as the time lag between the spawning of the males and females was greater than the three hours considered to be the limit for successful fertilisation (Kikuchi and Uki, 1974b). The eggs which did fertilise developed normally through trochophore and post-torsion veliger, but as the numbers were so small, (< 5000) a set was not attempted.

Spawning attempts with intragonadal and intramuscular Serotonin injections were unsuccessful, and did not even induce pre-spawning behaviour. The most successul and reliable method proved to be a combination of hydrogen peroxide baths and thermal shock. Ultraviolet irradation stimulation was successful, but problems controlling the very low flow rates needed to achieve the required irradation (Grant, 1977) caused spawning schedules to be difficult to predict.

Intermittent water quality problems encountered at Tasmanian Shellfish Company caused several problems, notably:

a. Low salinities led to the rapid rupturing of the vitelline membrane of otherwise viable eggs.

b. Low salinities and high temperatures resulted in brood stock deaths.

c. The spawning of sperm which was not motile was observed for the first time, possibly as a result of stress induced by (b).

2. Gear Evaluation

On-growing techniques employed by John McMullen, Ab Lab, California were used as a basis for developing an approach suitable for Tasmanian application. His technique utilises modified 220 litre polyethylene drums suspended from an old jetty in a U.S. Navy base, and has proved a reliable method over several years.

For these trials, twenty litre, black polyethylene drums were modified by replacing the tops and bottoms with 6 mm polyvinylchloride mesh and by removing 13% of the lateral surface of the drums by perforating them with 8 mm holes. Doty block trials indicated that these drums allowed 80% of available water movement, and later tests when the drums were stocked at their usual density (100 abalone and approximately 1 kg of seaweed per barrel) indicated that there was no difference in levels of dissolved oxygen inside and outside the drums.

Algae was supplied in excess of 20% of total abalone body weight per day, and fed every 7 - 14 days.

The drums were initially suspended from a mussel long-line at depths of two, three and four metres sub-surface. The abalone evidenced no growth under this regime during a 47 day period, and it was thought that they may have been subjected to undue stress due to the movement of the long-line in rough weather, as evidenced by their tangling in mussel drop-lines.

The drums were then moved to bottom anchors with sub-surface floats, located by lazy-lines to the long-line. Some algae species returned promising abalone growth rates under the new regime, but some distress would have been suffered by the abalone still, as the drums were found entangled in mussel drop-lines on several occasions.

The final anchoring system used at Nubeena was as above, except that the lines were located by a light line with a small float instead of a lazy-line, and were located well clear of potential entanglements. This system produced good growth over a 53 day trial, with growth rates of up to 70 microns per day recorded.

A variation of the previous anchoring system was used for trials at Bicheno. A galvanized steel chain was laid between two anchors on the bottom, and the drums, with sub-surface floats but without the locating floats, were attached to this instead of individual anchors as previously. The drums were now set at depths of seven and nine metres.

Although Bicheno had shown in previous small trials to give better growth rates than Nubeena, no abalone under the above regime showed a growth rate in excess of ten microns per day during an initial 82 day trial. As it was thought 82 days was sufficient for the trauma of the transfer from Nubeena to Bicheno to have dissipated, the trial was modified to determine the cause of the cessation of growth.

1. A drum containing juvenile abalone collected from nearby sea bottom was added to determine whether the hatchery produced abalone were suffering long term effects of previous continued handling.

2. A galvanized steel cage, with a PVC mesh cover, fixed to the sea floor and containing some naturally conditioned rocks as a substrate, was stocked with abalone from one of the container evaluation drums to give an indication as to whether the constant movement of the drums was contributing an effect. The cage allowed 95% of available water movement.

3. One drum was anchored in a tank at Shellfish Culture Pty. Ltd., providing excellent water quality and movement (aerated).

4. Hatchery produced and wild harvested abalone were returned to an aquarium at the laboratory.

5. Two drums were not brought to the surface during the course of the trial, but were fed underwater by divers, to investigate the effect of removal from the water for feeding on the abalone.

6. A container comprising six circular PVC plates set mutually perpendicular inside a PVC mesh covered stainless steel cylindrical frame was added to the long-line. This container permitted 97% of available water movement.

7. The feeding interval was extended to from 14 days to 35 days to minimize disturbance.

Results

Results of the 141 day trial were:

	GROWTH RATE (microns/day)	WEIGHT GAIN* (mgms/day)	MORTALITY (%/day)
M. pyrifera			
1. no disturbance	5.04	9.72	0.15
2. wild stock	5.56	1.35	0.03
3. mesh container	20.28	26.03	0.46[a]
4. bottom cage	15.81	10.95	0.36[a]
5. at hatchery	3.43	0.76	0.00
6. aquarium-wild stock	59.53		0.39
7. aquarium-hatchery stock	38.60		0.00
8. control	-4.04	15.32	0.15

* weights are influenced by, in some instances heavy, infestations of *Ostrea angasi*, and *Spirorbis* spp. and other polychaeta on the abalone shell.

[a] inflated by insecurity of the container.

It is valid to compare the mean figures for each treatment as the entire population was measured in every instance. Negative growth rates can be attributed to shell damage during handling.

A minimal improvement in growth rate was detected in the cages which were not lifted to the surface for each feeding. Both the mesh container and the bottom cage demonstrated improved growth rates compared to other containers in the trial, but these rates were still lower than the aquarium rates, and also the drum rates from previous trials.

The hatchery produced abalone grew at a much faster rate in the aquarium than on the long-line, and of the wild abalone used, the growth rate of the aquarium abalone was an order of magnitude faster than the long-line abalone. Peter Whyte (Pers. Comm. 1985) advised that year-long mean growth rates of hatchery produced abalone seeded in two sites (one exposed, one sheltered) were 47 and 82 microns per

day respectively. Harrison and Grant (1970) recorded growth rates of wild abalone, at a semi protected and an exposed site, of 88 and 101 microns per day respectively for the first year. While the growth rate of the wild abalone in the aquarium could be considered consistent with these results, the wild abalone on the long-line produced growth rates far below these recorded in natural populations.

Mortality rates in all trials were acceptable with the exception of the mesh container and the bottom cage. The mesh container was damaged in a storm and abalone escaped before repairs were effected. Abalone occasionally became wedged between the PVC mesh and the galvanized steel cage, and would then die. The high mortality experienced by the wild stock in the aquarium may be attributable to a combination of damage on collection and stress of the journey back to the laboratory.

Discussion

The results of the culture work conducted over the past year indict the long-line based barrel technique as unsuitable for application to many of the sites which are currently under consideration for abalone culture. Although it is not possible to specifically isolate the effect of the long-line system from the contributing influences of handling (Tong, 1985), preliminary work in the laboratory indicates that substrate movement may affect the growth rate of abalone. The mean consumption rates (expressed as a percentage of the mean body weight of the abalone per day) of abalone subject to substrate movement was less than half that of abalone under identical but static conditions. The experimental design needs further modification before reliable results can be obtained (eg. there is currently no facility to prevent build up of metabolic waste during the trials; Sano and Batei (1962) demonstrated that increases in ammonia levels result in decreasing feeding rates), but the initial results do reflect the trend observed in the field, where feeding rates in drums subject to entanglement or wave action were generally depressed.

Further work in this area is intended, especially in the measurement of mucous production. Peck (1983) calculated the calorific value of mucous of _H. tuberculata_ as 5436 cal/g dry weight; and Culley and Sherman (1985) demonstrated a relationship between mucous production and substrate particle size. A common view is that abalone secrete more mucous in reaction to increased stress, and if this occurrs in respect to substrate movement, a large portion of the abalone's energy budget may be utilized in this reaction.

3. Algae Evaluation

Blacklip abalone are described by Shepherd (1972) as opportunistic feeders, feeding mainly on drift algae, but resorting to browsing or grazing when food supplies are low. He contends they prefer red algae to brown or green, but do not select one red alga in preference to another; rather, they feed on available species in proportion to their abundance. Leighton (1964) found that abalone were capable of selective feeding, and that, with regard to three species of American abalone, *Macrocystis pyrifera* was a preferred diet. Harada and Kawasaki (1982) discovered the Japanese abalone, *Haliotis discus*, preferred brown algae to red or green. Lubchenco (1978) observed that, while herbiverous prosobranchs in aquaria do not appear to be able to detect food at a distance, those that can identify food by contact chemoreception are also capable of considerable food choice, selecting algae that lack structural or chemical deterrents to herbivory. Abalone do not always prefer food which results in fastest growth, however (Chen, 1984).

To provide a starting point for culture tests, adult abalone (length 90-135 mm) were collected from Safety Cove and Fortesque Bay on the Tasman Peninsula and analysed for gut contents. *Plocamium angustum*, *Corallina officinalis* and *Ballia callitricha* were positively identified in the guts, and *Jeanerettia lobata* and *Gelidium glandulaefolium* were present in the sampling area and may have been present in the guts as detritus.

A trial design to compare the suitability of these algae as abalone food was commenced at a trout farm at Nubeena, using some of the above algae, plus *M. pyrifera*, on which the abalone, produced in the hatchery under FIRTA 83/52, had been surviving since transfer from the nursery. Algae was provided at a rate of 20% of the total abalone body weight per day. Growth was recorded with all algal species except *C. officinalis*.

Due to the influence of the ever burgeoning trout farm, it was decided to move the on-growing trials to Waubs Bay at Bicheno. Two trials were conducted before transfer to compare the two sites, one using *P. angustum* and the other using *M. pyrifera*, under otherwise identical conditions to those of the last trial at Nubeena. After an initial period of slow growth, possibly due to the effects of the removal operation, growth rates at Bicheno showed an improvement of 4.45 and 5.13 microns per day respectively over the growth rates at Nubeena.

Phacelocarpus alatus, *Phyllospora comosa* and *B. callitricha* feeding trails were conducted at Bicheno.

Results

	GROWTH RATE[a] (microns/day)	WEIGHT GAIN[b] (mgms/day)	MORTALITY (%/day)
PHAEOPHYTA			
M. pyrifera[1]	26.98	10.75	0.16
M. pyrifera[2]	-4.04	15.32	0.15
P. comosa	6.17	11.63	0.08
RHODOPHYTA			
B. callitricha	5.11	3.83	0.14
C. officinalis	5.09	5.02	0.14
P. angustum	38.68	17.17	0.10
J. lobata	27.55	15.66	0.13
G. glandulaefolium	70.00	35.09	0.04
P. alatus	2.91	8.16	0.13

	SPECIFIC[c] GROWTH RATE (% body wt/day)	ALGAE CONSUMPTION (% body wt/day)	ALGAE[d] CONVERSION (algae wt/ab wt)
PHAEOPHYTA			
M. pyrifera[1]	0.61	14.22	23.60
M. pyrifera[2]	0.31	6.00	19.88
P. comosa	0.24	3.29	13.65
RHODOPHYTA			
B. callitricha	0.09	0.00	0.00
C. officinalis	0.01	0.64	62.70
P. angustum	0.71	8.97	12.78
J. lobata	0.68	3.00	4.48
G. glandulaefolium	1.07	3.02	2.91
P. alatus	0.18	2.65	14.85

a may be influenced by gear design (see Gear Evaluation).

b weights are influenced by, in some instances heavy, infestations of _Ostrea angasi_, and _Spirorbis_ ssp. and other polychaeta on the abalone shell.

c Specific Growth Rate = $\frac{100 \ln (N/N_0)}{t}$ where N = final weight (g) N_0 = initial weight (g), and t = elapsed time (days).

d Algae Conversion is the amount of an alga required to produce an increase of one gram in the weight of one abalone (wet weights). Growth of fouling organisms (b) would affect this figure.

1 Nubeena control.

2 Bicheno control.

The problems with the long-line technique, and the apparently cumulative effects of intensive handling, which became obvious at Bicheno unfortunately means that the results obtained from those trials may not be truly indicative of the food value of the relevant algae.

Growth rates obtained from the Nubeena trial exhibit a range from effectively zero (*C. officinalis*) to 70 microns per day (*G. glandulaefolium*). The mean growth rate required to grow abalone to 50 mm in two years (the envisaged scenario) is 69 microns per day.

Mortality rates with all algae species were acceptable, despite the poor food value of some species, and would probably be improved further when culture techniques are refined. Apparent discrepancies in mortality rates between good and poor food value alga (ie. *M. pyrifera* 0.16%/day; *P. comosa* 0.08%/day) can be attributed to escape and handling related mortalities.

B. callitricha, *C. officinalis*, *P. alatus*, *P. comosa* and *M. pyrifera*^{2} all returned very low growth rates. Except for *C. officinalis* all these alga were evaluated at Bicheno, where as comparison of *M. pyrifera*^{1} and *M. pyrifera*^{2} evidences, growth rates were generally depressed. Consumption rates of *B. callitricha* and *C. officinalis* were very low ($<1\%$ bw/day) indicating that juvenile abalone do not prefer these algae.

Of the several algae which were consumed at a moderate rate by the abalone in the trials, *P. angustum*, *J. lobata* and *G. glandulaefolium* returned mediocre to good length increments and specific growth rates, indicating that the abalone may have been growing at their maximum potential on these algae. *M. pyrifera*^{2}, *P. comosa* and *P. alatus*, however, precipitated moderate consumption rates, but evidenced very small length increments, similar to *B. callitricha* and *C. officinalis*. They are distingiushed from the latter two alga by their specific growth rates, which are much higher. As these three algae were all trialled at Bicheno, it is likely that abalone would grow much better on these algae, given better conditions.

The result of *C. officinalis* is particularly interesting as the gut contents of larger abalone (90-135 mm) comprised approximately 60% fresh and partially digested *C. officinalis*. A factor contributing to this would be the long gut retention time of this alga due to its calcerous cell wall, possibly exaggerating true volumes consumed. Nonetheless it seems that larger abalone may consume this alga regardless of its apparent poor food value.

Discussion

G. glandulaefolium, although only a small plant and present in only a very small biomass, returns rapid growth (both in shell length and specific growth) for small consumption rates. It's morphology appears more suitable for very small abalone, making it an ideal choice for weaning abalone from micro to macro aglae, a transition which has

previously been subject to problems. Gelidium sp. have been successfully cultured (Correa et al, 1985), as has Gracilaria sp. (Hansen, 1983). Gracilaria sp. is cultured for abalone food in new Zealand (Christeller et al, 1983) and Taiwan (Chen, 1984), and has been recorded in Tasmanian waters (Ferguson Wood, 1945). Culture of these agarophytes as an abalone food source may be feasible and preferable to wild harvesting, as well as having the potential to develop into industries in their own right (Hansen et al, 1981).

Although M. pyrifera returned only mediocre abalone growth rates during the field trials (31 microns per day), its abundance (Cribb, 1953) and rapid growth rate (Harger and Nueshel, 1983) select it as the only alga of those tested that could immediately be applied to large scale farming of abalone in Tasmania. Given the fact that the hatchery bred abalone, with which all the algae evaluations were conducted, exhibited a depressed growth rate in the field compared to in aquaria, and also compared to wild abalone under the same conditions, it is feasible that abalone growth rate on M. pyrifera could be greatly improved under more optimum conditions.

Artificial diets for abalone have been tried in various areas around the world. These have usually been based on seaweed or seaweed products such as sodium alginate, with protein, starch, yeast and sometimes vitamins added (Mottet, 1978b). A sorghum-based artificial food is currently being used in Japan, but it has been found that abalone require some leafy seaweed in addition to this. Growth rates and meat/shell weight ratios are claimed to be greatly improved under this regime (Cuthbertson, 1985).

REFERENCES

Chen,H., 1984. Recent innovations in cultivation of edible molluscs in Taiwan, with special reference to the small abalone Haliotis diversicolour and the hard clam Meretrix lusoria. Aquaculture, 39: 11-27.

Christeller, J., Furneaux, R., Gordon, M., Laing, W., Miller, I., Nelson, W., Shaw, B. and Terzaghi, B., 1983. The potential for mariculture of seaweeds in New Zealand. Tech. Rep. 18, DSIR.

Correa, J., Avila, M. and Santelices, B., 1985. Effects of some environmental factors on growth of sporelings in two species of Gelidium (Rhodophyta). Aquaculture, 44: 221-227.

Cribb, A., 1954. Macrocystis pyrifera (L.) Ag. in Tasmanian waters. Aust. J. Mar. Freshwat. Res. 5 (1): 1-34.

Culley, M. and Sherman, K. 1985. The effect of substrate particle size on the production of mucous in Haliotis tuberculata L. and the important of this in a culture system. Aquaculture, 47: 327-324.

Cuthbertson, A.S., 1985. Personal communication.

Ebert, E. and Houk, J., 1984. Elements and innovations in the cultivation of red abalone Haliotis rufescens. Aquaculture, 39: 375-392.

Ferguson Wood, E., 1945. The sources of agar in Australia. J. Council Sci. Ind. Res. 18 (3): 263-75.

Gibbons, M. and Castagna, M., 1984. Serotonin as an inducer of spawning in six bivalve species. Aquaculture, 40: 189-191.

Grant, J., 1981. Abalone culture in Japan: Development and current commercial practice. Tas. Fish. Res. 23: 1-17.

Hansen, J., 1983. A physiological approach to mariculture of red algae. J. World Maricul. Soc. 14: 380-391.

Hansen, J., Packard, J. and Doyle, W., 1981. Mariculture of red seaweeds. Rep. No. T-CSGCP-002 cal. Sea Grant Col. Prog. Pub.

Harada, K. and Kawasaki, O., 1982. The attractive effect of seaweeds based on the behavioural responses of young herbivorous abalone Haliotis discus. Bul. Jap. Soc. Sci. Fish. 48 (5): 617-621.

Harger, W. and Neushul, M., 1983. Test - farming of the giant kelp, Macrocystis, as a marine biomass producer. J. World Maricul. Soc. 14: 392-403.

Harrison, A. and Grant, J., 1970. Progress in abalone research. Tas. Fish. Res. 5 (1): 1-10.

Kikuchi, S. and Uki, N., 1974a. Technical study on artificial spawning of abalone, genus Haliotis. II. Effect of ultraviolet irradiated seawater to induce spawning. Bull. Tohoku Reg. Fish. Lab., No. 33: 80-92 (in Japanese with English abstract).

Kikuchi, S. and Uki, N., 1974b. Technical study on artificial spawning of abalone, genus Haliotis IV. Duration of fertility related to temperature. Bull. Tohoku Reg. Fish. Lab., No. 34: 73-75 (in Japanese with English abstract).

Leighton, D., 1964. Studies of food preference in algivorous invertebrates of Southern California kelp beds. Pacif. Sci. 20 (1): 104-113.

Lubchenco, J., 1978. Plant species diversity in a marine intertidal community: Importance of herbivore food preference and algal competitive abilities. Am. Nat. 112: 23-39.

Morse, D., Duncan, H., Hooker, N. and Morse, A., 1977. Hydrogen peroxide induces spawning in molluscs, with activation of prostaglandin endoperoxide synthetase. Science 196: 298-300.

Mottet, M., 1978a. A review of the fishery biology of abalones. Washington Dept. of Fish. Tech. Rep. 37: 30.

Mottet, M., 1986b. A review of the fishery biology of abalones. Washington Dept. of Fish. Tech. Rep. 37: 23-24.

Peck, L., 1983. An investigation into the growth and early development of the ormer, Haliotis tuberculata. Ph.D. Thesis (N.A.A.), Portsmouth Polytechnic, Portsmouth.

Sato, T., and Batei, R., 1962. Environmental conditions affecting the growth of Ezo-awabi. Bull. Tohoku Fish. Exp. Stat. 21: 79-86.

Shepherd, S., 1973. Studies on southern Australian abalone (genus Haliotis) 1. Ecology of five sympatric species. Aust. J. Mar. Freshwat. Res. 24: 217-57.

Thomson, T., 1983. Current abalone techniques in Japan. B.C. Shell. Maricul. Newsletter, Brit. Col. Min. Envir., 3(5): 13-16.

Tong, L., 1985. Personal communication.

Whyte, P., 1985. Personal communication.

Young, J. and DeMartini, J. The reproductive cycle, gonadal histology, and gametogenisis of the red abalone Haliotis rufescens (Swainson). Calif. Fish Game, 56 (4): 298-309.

84/050

Embryonic and larval development of the Blacklip Abalone, *Haliotis ruber* (Leach, 1814).

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Abstract

Eggs of *H. ruber* kept at 15.9°C commenced hatching as trocophores 17 hours 30 minutes after fertilisation. Hatching was complete 1 hour 10 minutes later. Torsion took place between 43 and 45 hours after fertilisation and larval shells were complete at 47 hours. Veligers at this time showed budding cephalic tentacles, a foot, mantle, operculum and eye spots. Appearance of the first epipodial tentacle and cilia of the ctenidium at 106 hours is for some northern hemisphere species indicative of impending settlement, however, settlement of *H. ruber* did not occur for a further 36 hours.

INTRODUCTION

Studies of the reproductive biology of Australian species of the marine gastropod genus *Haliotis* have been limited to work by Harrison and Grant (1971) and Shepherd and Laws (1974) on adult reproductive stages.

In 1980 an experimental abalone hatchery was established at the Tasmanian Fisheries Development Authority's Research Laboratories to develop suitable hatchery techniques for production of juvenile blacklip abalone.

Several spawnings have been induced. Close monitoring of development for 150 hours from fertilisation has been possible.

Our paper details the previously unknown early development of *Haliotis ruber.*

MATERIALS AND METHODS

Brood stock were selected at sea from commercial abalone diver's catches. The criterion for selection was visible gonad bulk size. Sex is readily determined by manipulating the mantle and observing the colour of the gonad: males are cream to white, females green or grey.

Ten males and ten females were transported directly to the laboratory in a standard polypropylene draining fish box. Abalone were in two layers separated by clean plastic onion bag mesh and covered with a two inch layer of fresh algae *(Phyllospora spp.)*. The interval between collection and return to the hatchery was two hours ten minutes. After gently scrubbing the shells under running seawater two females and two males were placed separately into each of ten, 50 litre clear acrylic tanks filled to 40 litres with 1 μm filtered seawater at ambient 16°C. A flow rate of 1 litre per minute was maintained. After one hour at ambient the temperature was raised 3.5°C during the next hour and H_2O_2 was added to each tank to an initial concentration of 0.25 mM (Tanaka, 1978).

The temperature was maintained at 19.5°C for two hours then returned to ambient by natural cooling. Six hours fifteen minutes

from immersion one male commenced spawning, however, no further spawning activity was observed for eight hours fifteen minutes when six males and one female commenced spawning within fifteen minutes. Eggs and sperm released into the branchial cavity were forcibly ejected through the second and third respiratory pores. Unfertilised eggs are usually brownish but as with *H. discus hannai* (Grant, 1981) they may occasionally vary to green or grey. The yolk diameter is 200 µm including a vitelline membrane 18 µm thick and is surrounded by a gelatinous mass roughly spherical in shape. Total diameter is 260 µm (Fig. 1).

Eggs were filtered through a 353 µm sieve to remove faeces and mucus and the water changed once by decanting and refilling.

A sperm count showed a concentration of 2.2×10^6 per ml in the spawning container. Sperm water was added to the eggs to produce a concentration of 2×10^5 per ml (Kikuchi and Uki, 1974) and allowed to stand for ten minutes. Eggs were washed a further seven times at ten minute intervals to remove excess sperm. Until development of a larval shell further handling was considered undesirable. Once the shell had formed, providing shelter for the veligers, draining cleaning and refilling of larval culture tanks was considered safe. Larvae were siphoned on to a partly submerged 85 µm mesh sieve during this operation. Larvae were in this way unexposed to air during water changes which occurred at least twice a day.

Water temperatures throughout the period of development ranged between 15.6°C and [illegible]°C.

SPAWNING AND DEVELOPMENT

The first polar bodies were observed 40 minutes after fertilisation (Fig. 2). First cleavage was noted 110 minutes after

fertilisation and takes place meridionally. Division of the egg was complete and unequal as described for *H. sieboldii*, *H. discuss*, *H. tuberculata* and *H. gigantea* (Ino, 1952) (Fig. 3).

At this stage fertilisation rate was 91% with 5% unfertilised and 4% developing abnormally. Second cleavage occurred at 2 hours and 25 minutes post fertilisation, third at 3 hours, 23 minutes and the fourth at 4 hours, 12 minutes. The morula stage was reached at 8 hours, 35 minutes (Fig. 4) and early gastrula (Fig. 5) at 9 hours, 10 minutes. The earliest trocophore stage was reached at 13 hours when cilia were observed on the prototrochal girdle. First rotation within the egg membrane occurred at 13 hours, 17 minutes and 15 hours after fertilisation all trocophores were actively rotating. At 17 hours, 15 minutes crinkling of the egg membrane was evident and 15 minutes later the first trocophore was observed to burst out and swim vigorously (Fig. 6). Hatching activity increased rapidly and reached a peak after a further 6 minutes. Hatching was complete at 18 hours, 40 minutes (Fig. 7). Larvae were observed all through the water column (Fig. 8) and actively massed on the surface forming in vertical columns and spontaneously tumbling to the bottom continuously dispersing and reassembling, a phenomenon which indicates normal healthy larvae (Grant, 1981).

Larval shell growth had commenced by 29 hours, 45 minutes (Fig. 9) and torsion, 90° twisting of the cephalo-pedal mass within the larval shell, took place from 40 to 45 hours.

After 47 hours fertilisation larvae shells were complete, veligers showed budding cephalic tentacles, a foot, mantle, operculum and eye spots (Fig. 10). The larvae retracted into the fully developed larval shells and rested intermittently.

Development slowed between 50 and 100 hours after fertilisation and only a slight enlargement of the foot and extension of cephalic tentacles was observed. The protrusion of the first epipodial tentacle and cilia of the ctenidium were identified at 106 hours. The appearance of these features are considered for some northern hemisphere species to be indicative of impending settlement (Grant, 1981), however, settlement of *H. ruber* did not occur for a further 36 hours.

Larvae were settled onto corrugated green plastic plates which had been immersed in the sea for three weeks to collect a coating of diatoms on which post settlement larvae would feed.

At 143 hours no larvae were observed still swimming. Examination of section of the settlement plate revealed actively crawling larvae with shells in an upright position. The cilia on the prototrochal girdle were active and if disturbed the larvae would retract into the shell and topple over, resting for about 30 seconds before emerging and righting the shell once more.

Settlement plates were monitored for a further four days during which time larvae displayed grazing behaviour and shells, displaced into the horizontal plane, showed post settlement growth.

Anatomical development of *H. ruber* followed closely that of *H. sieboldii* as described by Ino (1952). Temperature during development was similar and the rate of development until the late larval stage also matched closely. *H. sieboldii* reached early creeping stage at 120 hours, however *H. ruber* remained actively swimming for a further 22 hours before settlement.

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REFERENCES

Grant, J.F. (1981): Abalone culture in Japan: Development and current commercial practice. *Tasmanian Fish. Res.* No. 23, pp. 1-17.

Harrison, A.J. and Grant J.F. (1971): Progress in Abalone Research. *Tasmanian Fish. Res.* 5(1), pp. 1-10.

Ino, T. (1952): Biological studies on the propagation of Japanese Abalone (Genus: Haliotis). *Bull. Tokai Fish. Res. Lab.* No. 5, 102 p.

Kikuchi, S. and Uki, N. (1974): Technical study on artificial spawning of Abalone, Genus Haliotis III. Reasonable sperm density for fertilisation. *Bull. Tohoku Reg. Fish. Res. Lab.* No. 34, pp. 67-71.

Shepherd, S.A. and Laws, H.M. (1974): Studies on Australian abalone (Genus Haliotis) II. Reproduction of five species. *Aust. J. Mar. Freshwater Res.* 25(1), pp. 49-62.

Tanaka, Y. (1978): Spawning induction of the Abalone *Nordotis gigantea* by chemical control with hydrogen peroxide. *Bull. Tokai Reg. Fish. Res. Lab.* No. 3[illegible], pp. 93-10[illegible].

Fig. 1. Unfertilised egg. Diameter including egg membrane is 260 µm. Scale 100 µm.

Fig. 2. Fertilised egg showing polar bodies (arrow).

Fig. 3. First cleavage. 1 hour, 50 minutes.

Fig. 4. Morula stage (right) and slower developing 16 cell stage (left). 8 hours, 35 minutes.

Fig. 5. Early gastrula stage. [illegible] hours, [illegible] minutes.

Fig. 6. Trocophore bursting through the egg membrane (right) and hatched free swimming trocophore (left). 17 hours, [illegible] minutes.

Fig. 7. Hatching complete. Discarded egg membranes and a swimming trocophore larva. Scale 300 µm.

Fig. 8. Free swimming trocophore larvae at 24 hours showing cilia of the prototrochal girdle (p.t.g.) and Apical tuft (a.t.)

Fig. 9. Trocophore at 29 hours, 45 minutes showing development of larvae shell (l.s.).

Fig. 10. Veliger with fully developed larval shell. [illegible] hours.