No 19.80./..16..... 80/016

- ☐ NEW PROPOSAL
- ☐ CONTINUING PROJECT
- ☒ FINAL REPORT
- ☐ PROGRESS REPORT

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TLE OF PROPOSAL/PROJECT: Experimental hatchery production of blacklip abalone, Haliotis ruber.

RGANISATION: Tasmanian Fisheries Development Authority

RSON(S) RESPONSIBLE: Mr. C.E. Sumner, Mr. J.F. Grant, Dr. T.G. Dix

UNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1980/81		$10,900

RELATED APPLICATIONS:

..31./..12./19.81 DISTRIBUTED ..9./.2.../1982

.....Judith Lowe.....
for Secretary
Fishing Industry Research Council

F139—12/81

80/16

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

FINAL REPORT

1981

ITEM

1. Title of Proposal: Experimental hatchery production of blacklip abalone, *Haliotis ruber*.

2. Name of Applicant: Tasmanian Fisheries Development Authority.

3. Division, Department or Section: Research and Resource Section.

4. Proposal: To develop techniques for the hatchery production of juvenile blacklip abalone.

5. Name of Persons Responsible for Programme:

   Mr. Colin E. Sumner, B.Sc. (Hons) - Marine Biologist

   Mr. John F. Grant - Senior Technical Officer

   Dr. Trevor G. Dix, Ph.D., B.Sc. (Hons) - Senior Marine Biologist.

6. Qualifications of Staff to be Employed on the Programme:

   C. E. Sumner, Marine Biologist, 8 years experience in mollusc culture. Recipient (1978) of French Government Professional and Technical Scholarship to study advances in French mollusc culture including abalone.

   J. F. Grant, Senior Technical Officer, 12 years experience with mollusc research programmes. Recipient (1977) of Overseas Fishing Cooperation Foundation of Japan Scholarship to study abalone culture in Japanese research laboratories and commercial hatcheries. Two years experience with the management of a successful pilot oyster hatchery at the T.F.D.A. laboratory.

H. Tyler (Cadet technician, aquaculture) 12 months experience at T.F.D.A. oyster hatchery.

7. Location of Operation: Base of operations will be the Research and Resource Section of the Tasmanian Fisheries Development Authority at Taroona in Tasmania. Abalone will be collected at various sites around Tasmania.

8. Proposed commencement date: July 1980

9. Proposed completion date: June 1981

10. Funds Requested:

	1980/1981
(a) Total salaries and wages	Nil
(b) Total operating expenses	$5,250.00
(c) Total capital items	$5,650.00
Gross total cost:	$10,900.00

11. Funds to be provided by the Applicant or sought from other sources:

The T.F.D.A. paid the salaries of all personnel. Capital equipment supplied and used at Taroona included water pumping, sand filtration and storage facilities and temperature monitoring gear. Laboratory facilities particularly microscopic and histological were utilised fully.

12. Variations in expenditure:

Expenditure closely followed that proposed there being a minor surplus of $641.16 in the account at completion of project.

13. Expenditure:

	1980/1981
Operating expenses	$4,769.04
Capital costs	$5,489.80
TOTAL	$10,258.84

Capital Items

	$
20 - Rectangle tanks	521.70
1 - Large tank	405.00
Water Sterilizer	2,100.00
550w pump and motor	244.50
4 - Mercury Thermometers	66.00
1 - Tank 110 x 150 x 62	266.00
Vitreous water heaters	84.00
Electrical Temperature Control	1,336.00
Filtrite prefilter pump & motor	264.00
Universal lead lag ballast	101.80
Transformer to suit sterilizer	101.80
	$5,489.80

14. Report:

During the period June 1980 to July 1981, eleven spawnings were attempted with brood stock collected from diver's catches in south eastern Tasmania. Spawnings of both sexes was induced successfully on six occasions by stimulating gravid abalone with an increase in water temperature and immersion in water irradiated with ultra violet light, a reliable Japanese developed technique. Three spawnings were unsuccessful probably due to poor condition

and bad handling of brood stock, one was partly successful with males only induced to spawn and one spawning spontaneously occurred uncontrolled in a holding tank with males and females spawning profusely.

As the project progressed it became increasingly apparent by inspecting diver's catches that gravid abalone could be found continuously throughout the year and conditioning adults in the laboratory would not be necessary. Due to a series of equipment failures and water supply problems this section of the project was then abandoned.

A reliable method of assessing maturity visually was developed. The mantle and foot were manipulated to expose the gonad which if markedly swollen, blunt and rounded at the tip indicated that the abalone was gravid. The developing gonad is quite pointed at the tip.

From the six successful spawnings three broods of juvenile abalone have been reared and their growth monitored. Embryonic and larval development was studied and photographed and a paper describing this segment is currently in press (copy attached).

Settled juveniles were acclimatised to a running sea water system at ambient temperature.

Three broods of larvae were monitored for settlement and immediate on-growing. Dietary components included wild benthic diatoms and cultured algae including the diatoms *Phaeodactylum tricornutum*, *Tetraselmis suecica* and *T. chuii*, *Navicula sp.* and *Nitzchia sp.* Larval density, food density, surface area type, and culture conditions were modified in an attempt to produce optimum conditions for settlement and metamorphosis.

The use of GABA, a chemical inducer found in naturally occurring coralline crustose red algae (Morse *et al.* 1979) appeared to enhance settlement rate and rate of metamorphosis. However settlement success rate as measured by percentage of pedi-veliger larvae removed to setting tanks surviving to formation of first respiratory pore was low (1%). Factors responsible for this poor survival include: settlement substrate type, green PVC corrugated plates were unsuccessful; algal food type and density, larvae appear to prefer low densities of small (<15 µm diameter) centric diatoms; and presence of pathogenic bacteria (*Vibrio sp.*) on settlement surfaces. Most of these factors can be manipulated successfully and it is proposed to accommodate new settlement procedures in future work.

In total over 2,000 creeping juvenile blacklip abalone were produced. Growth rates of newly settled abalone varied depending on culture circumstances but were in the range 15 µm/day rising to 50 µm/day on appearance of the first respiratory pore. Abalone formed pores around lengths 1.5 - 2.0 mm. This appears to be an important biological event in the developing juvenile. Feeding on certain macroalgae was noticed after this and mortality rates dropped. These latter studies are continuing as part of a FIRTA (1981/83) Grant.

Summary

Prospects for large-scale production of juvenile blacklip abalone have been enhanced by this study. Gonadal development, spawning inducers, larval handling and biology are now well documented. Settlement success rate remains low but continuing trials should lead to improvements.

Growth and survival of early creeping juveniles likewise should be improved. A stock of juvenile abalone have been produced which are now available for nursery culture experimentation and field on-growing.

Acknowledgements

We would like to thank Glenn and Ivan Cleaver for their co-operation in collection of adult brood stock for this project.

References

Morse, D.E., Hooker, N., Duncan, H., and Jensen, L. 1979. γ-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science 204* : 407-410.

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

John F. Grant and Colin E. Sumner,
Tasmanian Fisheries Development Authority,
Research Laboratory, Crayfish Point, Taroona, Tasmania, Australia.

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 40 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 363 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 Oki, 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 15.0°C and 16°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.

ACKNOWLEDGEMENTS

Assistance with the collection of brood stock by professional fishermen, Ivan and Glen Cleaver, is gratefully acknowledged.

H. Tyler assisted with hatchery work. The experimental abalone hatchery was supported in part by a Fishing Industry Research Trust Account Grant (1980/81). Dr. Trevor Dix reviewed the manuscript.

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. 96, pp. 93-103.

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. 9 hours, 10 minutes.

Fig. 6. Trocophore bursting through the egg membrane (right) and hatched free swimming trocophore (left). 17 hours, 30 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. 47 hours.

H. Tyler (Cadet technician, aquaculture) 12 months experience at T.F.D.A. oyster hatchery.

7. Location of Operation: Base of operations will be the Research and Resource Section of the Tasmanian Fisheries Development Authority at Taroona in Tasmania. Abalone will be collected at various sites around Tasmania.

8. Proposed commencement date: July 1980

9. Proposed completion date: June 1981

10. Funds Requested:

	1980/1981
(a) Total salaries and wages	Nil
(b) Total operating expenses	$5,250.00
(c) Total capital items	$5,650.00
Gross total cost:	$10,900.00

11. Funds to be provided by the Applicant or sought from other sources:

The T.F.D.A. paid the salaries of all personnel. Capital equipment supplied and used at Taroona included water pumping, sand filtration and storage facilities and temperature monitoring gear. Laboratory facilities particularly microscopic and histological were utilised fully.

12. Variations in expenditure:

Expenditure closely followed that proposed there being a minor surplus of $641.16 in the account at completion of project.

13. Expenditure:

	1980/1981
Operating expenses	$4,769.04
Capital costs	$5,489.80
TOTAL	$10,258.84

Capital Items

	$
20 - Rectangle tanks	521.70
1 - Large tank	405.00
Water Sterilizer	2,100.00
550w pump and motor	244.50
4 - Mercury Thermometers	66.00
1 - Tank 110 x 150 x 62	266.00
Vitreous water heaters	84.00
Electrical Temperature Control	1,336.00
Filtrite prefilter pump & motor	264.00
Universal lead lag ballast	101.80
Transformer to suit sterilizer	101.80
	$5,489.80

Total 90,030

14. Report:

During the period June 1980 to July 1981, eleven spawnings were attempted with brood stock collected from diver's catches in south eastern Tasmania. Spawnings of both sexes was induced successfully on six occasions by stimulating gravid abalone with an increase in water temperature and immersion in water irradiated with ultra violet light, a reliable Japanese developed technique. Three spawnings were unsuccessful probably due to poor condition

and bad handling of brood stock, one was partly successful with males only induced to spawn and one spawning spontaneously occurred uncontrolled in a holding tank with males and females spawning profusely.

As the project progressed it became increasingly apparent by inspecting diver's catches that gravid abalone could be found continuously throughout the year and conditioning adults in the laboratory would not be necessary. Due to a series of equipment failures and water supply problems this section of the project was then abandoned.

A reliable method of assessing maturity visually was developed. The mantle and foot were manipulated to expose the gonad which if markedly swollen, blunt and rounded at the tip indicated that the abalone was gravid. The developing gonad is quite pointed at the tip.

From the six successful spawnings three broods of juvenile abalone have been reared and their growth monitored. Embryonic and larval development was studied and photographed and a paper describing this segment is currently in press (copy attached).

Settled juveniles were acclimatised to a running sea water system at ambient temperature.

Three broods of larvae were monitored for settlement and immediate on-growing. Dietary components included wild benthic diatoms and cultured algae including the diatoms *Phaeodactylum tricornutum*, *Tetraselmis suecica* and *T. chuii*, *Navicula sp.* and *Nitzchia sp.* Larval density, food density, surface area type, and culture conditions were modified in an attempt to produce optimum conditions for settlement and metamorphosis.

The use of GABA, a chemical inducer found in naturally occurring coralline crustose red algae (Morse *et al.* 1979) appeared to enhance settlement rate and rate of metamorphosis. However settlement success rate as measured by percentage of pedi-veliger larvae removed to setting tanks surviving to formation of first respiratory pore was low (1%). Factors responsible for this poor survival include: settlement substrate type, green PVC corrugated plates were unsuccessful; algal food type and density, larvae appear to prefer low densities of small (<15 µm diameter) centric diatoms; and presence of pathogenic bacteria (*Vibrio sp.*) on settlement surfaces. Most of these factors can be manipulated successfully and it is proposed to accommodate new settlement procedures in future work.

In total over 2,000 creeping juvenile blacklip abalone were produced. Growth rates of newly settled abalone varied depending on culture circumstances but were in the range 15 µm/day rising to 50 µm/day on appearance of the first respiratory pore. Abalone formed pores around lengths 1.5 - 2.0 mm. This appears to be an important biological event in the developing juvenile. Feeding on certain macroalgae was noticed after this and mortality rates dropped. These latter studies are continuing as part of a FIRTA (1981/83) Grant.

Summary

Prospects for large-scale production of juvenile blacklip abalone have been enhanced by this study. Gonadal development, spawning inducers, larval handling and biology are now well documented. Settlement success rate remains low but continuing trials should lead to improvements.

Growth and survival of early creeping juveniles likewise should be improved. A stock of juvenile abalone have been produced which are now available for nursery culture experimentation and field on-growing.

Acknowledgements

We would like to thank Glenn and Ivan Cleaver for their co-operation in collection of adult brood stock for this project.

References

Morse, D.E., Hooker, N., Duncan, H., and Jensen, L. 1979. γ-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science 204* : 407-410.

80/16
7422y

Experimental Hatchery Production of Blacklip Abalone Haliotis ruber

Final Report

Organisation:

Tasmanian Fisheries Development Authority.

Staff:

Mr C.E. Sumner, Mr J.F. Grant and Dr T.G. Dix.

- Staff are highly competent and have undertaken a series of successful FIRTA - funded projects on shellfish culture techniques and also on abalone
  - this project brought two strands of research together.
- Objectives have been satisfactorily achieved with a moderate degree of success
  - further production of larvae should become routine
  - techniques now refined and pitfalls overcome
  - opens the way to mass production of larvae
  - now must investigate mass rearing of settled juveniles.
- Two avenues for industry development
  - culture through to adult of commercial size
  - wild population enhancement
  - both some way off.
- Currently a FIRTA-funded programme by TFDA (81/42)
  - "Shell fish aquaculture research and development"
  - carring work further using successfuly hatched larvae
- Scientific paper describing embryonic and larval development in press
  - has also been interim extension paper in June 1981 Australian Fisheries describing techniques

- papers describing techniques will undoubtly be published

- and seminars for interested fishermen and aquaculturalists will be held once techniques are further defined

- this is part of TFDA directive.