Short communication

Releases and recaptures of Manila clams (*Ruditapes philippinarum*) introduced to Norway

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The Manila clam, *Ruditapes philippinarum*, was introduced into Norway in 1987 and used in aquaculture until 1991. In 1988-91, spat were seeded on five Norwegian beaches in three regions at 60°N, 64°N and 65°N. Manila clams were recovered from three sites, at, or near where they had been released. They were distinguished from native *R. decussatus*, *Tapes pullastra*, and *T. aurea* using morphological features, confirmed by comparison of polymorphic isozyme loci using starch gel electrophoresis.

Fifty-one specimens were recovered, were 31-59 mm in shell length and ranged from 4 to 7 years of age, and were in good condition. Age of the recovered clams coincided with the time of the spat releases. Histological examinations revealed eggs and sperm in different stages of maturity, including spent ovaries, suggesting that spawning have taken place. No juvenile or small specimens were found.

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INTRODUCTION

The originally Asian Manila clam, *Ruditapes philippinarum* (Adams & Reeve, 1850) was accidentally introduced into British Columbia from Japan with imports of Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) seed (Quayle 1941). Following its introduction and establishment, the species became cultivated on the Pacific coast of Canada and the USA. It was later introduced into Europe, and through spreading after intertidal bottom culture, wild populations have been established also in several European coastal areas (Bourne 1982; Chew 1990; Flassch & Leborgne 1992; Sbrenna & Campioni 1994).

The Manila clam was introduced to Norway in 1987. The broodstock of approximately 500 specimens were imported from a Scottish hatchery to two Norwegian hatcheries. In one of these, the seed were grown in a land-based nursery using a landlocked marine basin (poll) as water and food source (Strand 1996). Over the period 1987 to 1991, 200 million seed were produced, and most of this was exported to Spain and Ireland.

In contradiction of both the procedures defined in the *ICES Code of Practice on the Introductions and Transfers of Marine Organisms* (ICES 1995) and Norwegian regulations (Anon. 1991) two permissions for experimental ongrowing of Manila clams were given by the Norwegian Directorate of Fisheries in 1989. Additionally, Manila clam spat were released without permission on at least four sites between 1988 and 1990. Neither release sites nor grow-out sites were however monitored, frames and trays which held the spat were abandoned, broken and washed away by the sea, and many of the Manila clams were left for an unknown destiny.

It was hypothesised that the Manila clam might have been established as a new species in the Norwegian fauna, and recruited to areas where cockles (*Cardium edule*), soft shell clams (*Mya arenaria)* and venerid clams (*Ruditapes decussatus*, *Tapes aurea*, and *Tapes pullastra*) are commonly found (Høisæter 1986; Wiborg & Bøhle 1974). The present work describes investigations at the release sites in 1995 and 1996, and a study of recovered clam specimens.

MATERIAL AND METHODS

Manila clam seed were cultivated at the following sites; 1: Espevik, Tysnes, in Hordaland county, 2: Vallersund in South Trøndelag county, 3: Rong, Øygarden, in Hordaland county, 4: Vågstranda, Møre and Romsdal county. There are six known release sites (Table 1). Surveys for the released clams were performed at four sites. The original seeding points were located, and all suitable substrates for clams were examined. The upper 30

cm of the substrate was removed in 1 m wide, 2-10 m long, transects and squares. All clams found were counted, marked and brought live to the laboratory.

Species were determined by both morphological features described by Cesari & Pellizato (1990) and Holme (1961), and starch gel isozyme analysis, using samples from the striated adductor muscle tissue in Tris-citrate-borate gel (TCB)(pH = 8.6) or tris-citrate II (pH = 8.0) buffer prepared for the enzymes malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), aspartate aminotransferase (AAT), D-octopine dehydrogenase (OPDH), phosphogluconate dehydrogenase (PGDH), Glucose-6-phosphate isomerase (GPI) and phosphoglucomutase (PGM).

Shell length was measured with callipers to the nearest mm, and shell and soft parts were drained on paper and weighed separately to the nearest 0.1 g. Age was determined from growth rings according to Johannessen (1973).

Transversal soft part sections were prepared according to standard procedures (Howard & Smith 1983). These were fixed in buffered 4 % formaline, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin/erythrosin/saffron (HES). Four sections from each specimen were examined in a light microscope at 25-400 × magnification.

RESULTS

Manila clams were found at Seløy, Vetle Godøy, and Aunvika (Table 1), together with cockles and clams of other species. All Manila clam shells were externally patterned brown on a cream-yellow background and had a distinct radial and concentric sculpture with a well defined lunule. The inner surface of all specimens from Seløy and Vetle Godøy was cream white, but with large areas with clear brownish and strong violet colour posteriorly, in the pallial sinus, extending anteriorly along the outer side of the pallial line. The pallial sinus reached into approximately 40 % of the shell length. No abnormalities were observed on the inner shell surface of any specimen. The Manila clams appeared to be in a good physiological condition. Gonadal tissues appeared full in most specimens, and gametes ran from gonads when cut or punctured. No abnormalities were observed in the soft-parts. Siphons were split. The results were confirmed by isozyme patterns, as IDH, AAT, OPDH, and PGDH clearly distinguished *R. philippinarum* from *R. decussatus*. MDH and IDH also distinguished these species from *T. pullastra* and *T. aurea* and the latter two from each other.

The Manila clams recovered from Seløy and Vetle Godøy were 43-60 mm and 38-51 mm respectively. All Manila clams from these sites were 7 years when recovered. The single live specimen found at Aunvika was 36 mm, 9 grams, and 4 years old. The distance from the last deposited concentric ring on the shell surface along the dorso-ventral axis to the shell margin was longer (mean of 1.3 mm) for clams from Vetle Godøy sampled in September, than from Seløy sampled in June (< 0.5 mm).

Histological examination revealed gonads in different developmental stages, through active to spent phases (Fig. 1). Differences in gamete development were observed both within the gonad of single specimens, and between specimens from the same site and sampling. In some specimens haemocytes filled with organic material, presumably from resorbed gonads, was observed in the stomach wall and in the connective tissue surrounding the stomach.

DISCUSSION

Clam species were easily distinguished by examination of shell and siphon morphology. Manila clams had a characteristic shell form, surface striation and coloration, clearly different from the other species. The determination of species was confirmed by the isoenzyme analysis. Clam species were distinguished by analysing for only a few enzyme systems, like IDH, MDH, and AAT with one buffer system. This was in accordance with Borsa & Thiriot-Quiévreux (1990) who reported that one locus, IDH2 was completely diagnostic for *Ruditapes philippinarum*, *R. decussatus*, and *R. aureus*.

The age of the Manila clams recovered coincided with the time reported for release. This agreement, and the

Table 1. Release and recapture sites of Manila clam *Ruditapes philippinarum* in Norway.

Location	Position		Year of release	Number released	Shell size (mm)	Time of survey	Number recaptured	Shell size (mm)
Seløy	59°55'N	5°43'E	1988	2000	-	June 1995	28	43-60
Vetle Godøy	60°05'N	5°35'E	1988	10 000	10-15	Sept 1995	22	38-51
Aunvika	64°16'N	10°23'E	1991	20-30 000	10-15	Dec 1995	1 (2)	36
Langsand	63°37'N	9°55'E	1988	2000	12-15	Aug 1996	0	-
Vallersund	63°50'N	9°40'E	1988-89	>2000	-			
Trellnes	65°22'N	12°10'E	1990	2000	-			

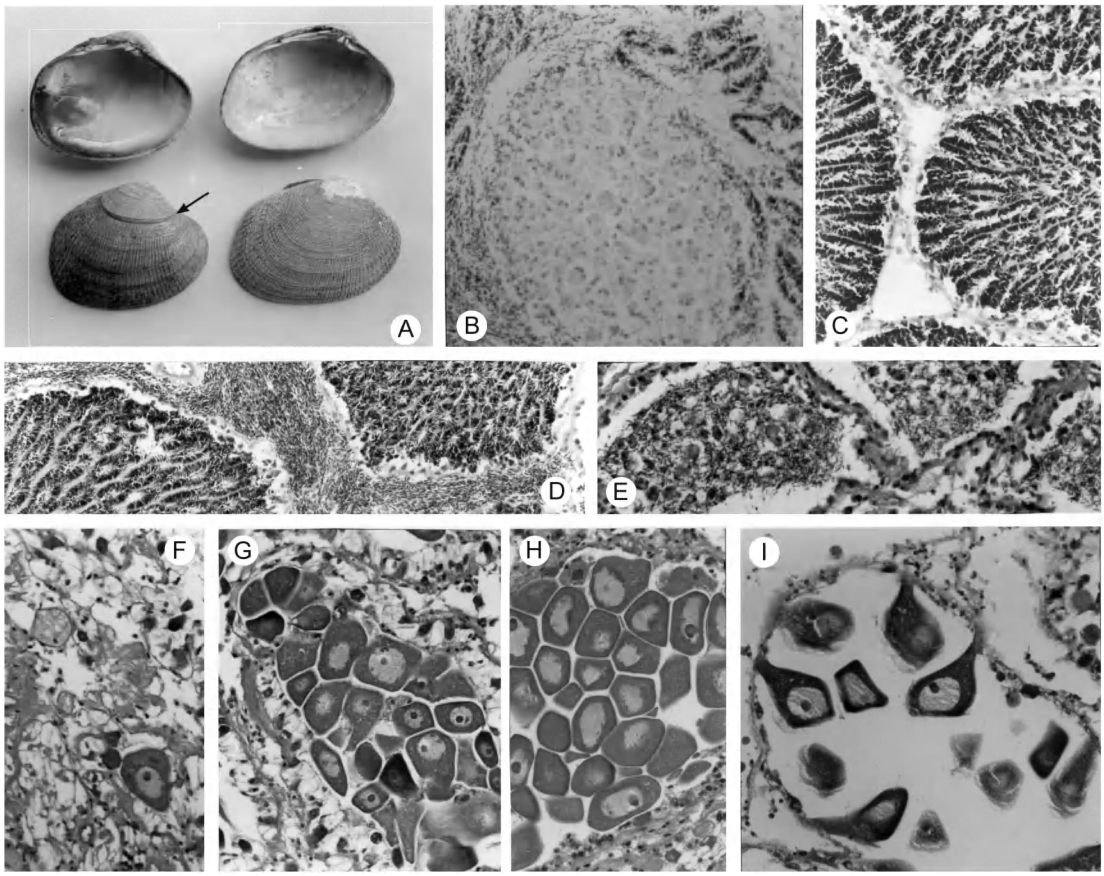

Fig. 1. A: Shells of Manila clam (left) and *Ruditapes decussatus*. Note the dark (brownish and violet) coloration on the inner shell of the Manila clam, in the pallial sinus and along the ventral side of the shell edge, as well as the condensed growth rings (arrowed). B-I: Histology of Manila clam gonad tissues. Original magnification 200 ×. B: Area in male gonad of the single live specimen recovered at Aunvika, containing distinct area packed with haemocytes. C and D: Mature male gonad in specimen from Vetle Godøy recovered in September, showing spermatozoa organised in rosette-like clusters. Free spermatozoa are visible between the two follicles in "D". E: Male gonad of specimen recovered at Seløy, in June. Resorption of residual spermatozoa by haemocytes in follicle lumina. F-H. Female gonad tissues of specimens recovered at Seløy. F: Inactive part of gonad, containing one single mature oocyte. G: Late active gonad with developing and mature oocytes in follicle. H: Mature oocyte. I: Spent female follicle of specimen recovered at Seløy, containing several residual gametes.

clear difference in distance between the most recent deposited concentric ring and the shell margin, supported the distinct deposition of annual winter rings on the shell surface. Distinct winter rings have also been measured on the shell surface of Manila clams from the temperate zone of Pacific Canada (Bourne 1982). The present results clearly indicate that the Manila clams found were all survivors of the released individuals.

Except for one live Manila clam and two shells found at Aunvika, all Manila clams in the present study were found at the southernmost release sites, Seløy and Vetle Godøy. In addition to the expected higher temperature at the lower latitude, these sites differ from the others by their location in the entrance to heliothermic polls (Strand 1996) where heated water is supplied to the sites during most of the year. The temperature of the poll water from spring to autumn might be up to 10 °C higher than the ambient fjord water. These sites are also well protected from strong wave action, while the other sites are rather exposed.

The Manila clams recovered beyond the release site at Vetle Godøy must have been moved by the tidal current in the channel between the poll and the fjord, while severe wave actions and sediment instability probably caused the movements of clams in Aunvika. This shows that the released Manila clams may have moved beyond

the sampled areas at the sites and were therefore not recovered in this survey. The sampling in the channels between the fjord and the polls at Seløy and Vetle Godøy covered more of the potential dispersion area than the sampling at the large beaches at Aunvika and Langsand. It is therefore likely that the proportion of Manila clams not recovered by our sampling were higher at Aunvika and Langsand than at Seløy and Vetle Godøy.

Observation of gross morphology as well as histological examination indicated that the clams were in good physiological condition. Successful sexual maturation in the Manila clam is considered to occur at temperatures from 15 to 21 °C (Parache 1982). The temperature in the polls are likely to be above 15 °C from late spring to autumn (Strand 1996), while coastal waters have temperatures above this level only during late summer. The mature gonad tissues of the clams at Seløy and Vetle Godøy indicate that successful spawning and probably dispersion of larvae have occurred from the sites. This is in accordance with observations made during histological examination of Manila clams held in the hatchery and nursery in Espevik in 1989-1992 (Mortensen 1993). As the present examination of gonads revealed gametes in different stages of development, including spent ovaries with residual mature eggs (Fig. 1I) it seems probable that spawnings have been asynchronous. This finding is in accordance with data reviewed by Ponurovsky & Yakovlev (1992) which show that spawning in Manila clams may occur throughout short or long periods of the summer season, depending upon location and environment, and may be continuous during the reproductive period.

Biological interactions such as predation may structure intertidal bivalve populations and be of major constraints to the farming of bivalves. The common shore crab *Carcinus maenas* has been identified as a main predator on seeded clams (Parache 1982). This crab was particularly abundant at Seløy, and may have caused high mortality of both the released clams and their potential offspring.

CONCLUSION

This present study of recovered Manila clams from release sites along the west coast of Norway showed that survivors of the seeded spat may appear in good physiological and reproductive condition. The status of gonads indicated that successful spawnings have occurred, and a spreading of larvae may have taken place over years. The spreading of Manila clams in several areas around the world (Chew 1990; Flassch & Leborgne 1992; Sbrenna & Campioni 1994), and in particular British Columbia (Quayle 1941; Bourne 1982), show that it could probably find favourable environments in some areas in Norway. No juvenile or small specimens were found in the present study, and there was thus no indication that Manila clam has been established in the Norwegian fauna. Considering the duration of the larval period, the potential offspring may have been carried by the currents to sites which were not covered by the present survey.

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