THE REPRODUCTIVE BIOLOGY OF THE DEPRESSED RIVER MUSSEL, *PSEUDANODONTA COMPLANATA* (BIVALVIA: UNIONIDAE), WITH IMPLICATIONS FOR ITS CONSERVATION

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ABSTRACT

Pseudanodonta complanata is listed as 'Near Threatened' on the IUCN Red List (IUCN, 2006) and is a species of conservation priority on the UK Biodiversity Action Plan. The UK is host to some of the largest populations of this species, but little is known about their reproductive biology. Two populations were studied in the Great Ouse catchment and the Waveney and Yare catchment, East Anglia, UK. Both populations are reproductively active, producing viable glochidia. Small mussels (<30 mm length) in both catchments indicate that recruitment is occurring. A short non-gravid period in May is followed by three months of glochidial formation in June, July and August; by September mussels contain mature glochidia ready for release the following April. Females brood between 5,000 and 50,000 glochidia, and this scales with mussel length L (mm) as: $n_{gloch} \propto L^{2.1}$. The sex ratio is skewed towards females (2.5 females:1 male), and males are larger than females. No hermaphrodites were found during the histological examination of the gonads of 24 mussels. The *in vivo* examination of demibranchs is shown to be an effective nonsacrificial means of determining sex and gravidity. Conservation recommendations include: minimizing management operations in river stretches containing large populations; avoiding forms of management which preferentially remove large mussels (i.e. males and the most fecund females); performing management during the non-gravid period to avoid causing the premature release of glochidia; leaving sufficient time between management operations for populations to recover; and temporarily translocating mussels to refugia during management operations.

INTRODUCTION

The precipitous decline in freshwater mussel populations has become a cause for concern in recent years (Bogan, 1993, 1998; Ricciardi, Neves & Rasmussen, 1998; Strayer *et al.*, 2004). In North America, of the 297 species once present, 12% are presumed extinct, and 60% are considered endangered or threatened (Ricciardi *et al.*, 1998). This trend is seen the world over (Bogan, 1993), and has been attributed variously to pollution, siltation, habitat destruction caused by impoundment, canalization and dredging, land-use change, loss of the obligate host-fish species, and the spread of invasive species such as the zebra mussel, *Dreissena polymorpha* (Bogan, 1993; Layzer, Gordon & Anderson, 1993; Neves, 1993; Williams *et al.*, 1993; Ricciardi *et al.*, 1998; Aldridge, 2000, 2004; Strayer *et al.*, 2004).

The depressed river mussel, *Pseudanodonta complanata* (Rossmässler, 1835) (Fig. 1), is a European freshwater bivalve which is currently listed as 'Lower Risk – Near Threatened' on the IUCN Red List of Threatened Species (IUCN, 2006). Its range extends across most of Europe, including Finland, Sweden, northern Russia, Austria, Germany, Switzerland, France, The Netherlands and the UK (Haas, 1969; Gittenberger *et al.*, 1998; IUCN, 2006). However, it is reported to be rare in most parts of this range (Schermer, 1935; Tudorancea, 1972; Haukioja & Hakala, 1974; Hüby, 1988; Englund & Heino, 1992; Willing, 1997; Kerney, 1999). In Germany, it is classed as 'threatened by extinction' and is afforded a high degree of protection by law (Der Bundesminister für Umwelt, Naturschutz und Reaktorsicherheit, 1986). In the UK, it has been included on the priority list of species of particular conservation concern in the Biodiversity Action Plan for the UK (Anon, 1995).

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There remains some confusion over the taxonomy of *P. complanata*. Haas (1969) recognized three geographically separated species: *P. elongata* Holandre, 1836, *P. complanata* Rossmässler, 1835 and *P. middendorffi* Siemaschko, 1849; he states that *P. elongata* is the form found in the UK. Current consensus holds that *P. complanata* is a single species (Falkner, Bank & von Proschwitz, 2001; Anderson, 2005), and we follow this classification here.

Despite evidence of a decline in UK populations in recent years (Müller, 1999), the UK is home to some of the largest populations of *P. complanata* in the world (Willing, 1997; McIvor, 1999; Müller, 1999). These populations are therefore of particular conservation importance, and they permit much-needed studies on the biology and ecology of *P. complanata*. Such studies are called for in the UK Species Action Plan for *P. complanata* (Willing, 1997), which states as one of its objectives: 'to initiate autecological research to develop a clearer understanding of . . . the life history of the species'.

Because most unionid species are relatively long-lived (between 10 and 100 years; Bauer, 1983; McIvor, 1999), relic populations may be found many years after recruitment has stopped, for example because of the loss of the host fish, a change in water temperature, or pollution affecting juvenile survival (Bauer, 1983, 1988; Heinricher & Layzer, 1999; Araujo & Ramos, 2000). Many such populations have been found (e.g. Chesney & Oliver, 1998; Heinricher & Layzer, 1999; Rogers, 1999; Kelner & Sietman, 2000; Morales *et al.*, 2004), and this emphasizes the importance of understanding the reproductive biology of a unionid mussel species in order to conserve it.

The reproductive biology of unionid mussels is unique: sperm are released by males into the water column to fertilize eggs being held in the outer demibranchs (gills) of females. The fertilized eggs develop into glochidia (larvae with bivalved shells)

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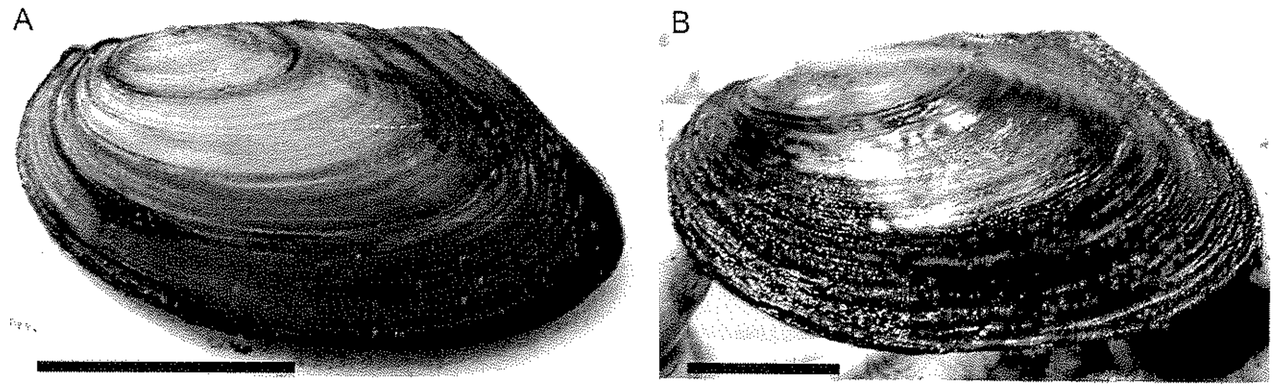

Figure 1. *Pseudanodonta complanata*, showing the forms found in the Great Ouse catchment **A** and in the Waveney and Yare catchment **B**. Scale bar = 20 mm. Photo **A** A. McIvor; photo **B** S. Müller.

within the females' gills, and are then released into the water column. To complete their development into juvenile mussels, they must parasitize certain host fish species, encysting on the gills, fins or scales for days or months. Having completed metamorphosis into juvenile mussels, they excyst from the host fish far from the parent mussel (Kat, 1984).

Previous studies have shown that *P. complanata* is a long-term winter brooder, holding glochidia for most of the year and releasing glochidia in spring or summer. This is followed by a short non-gravid interval, which is between March and May in Austria (Schierholz, 1889), in June in Finland (Pekkarinen, 1993) and between July and August in Russia (Zhadin, 1952). The only British study, based on six individuals from the River Cam (a tributary of the Great Ouse), found that all specimens were gravid in July (Aldridge, 1999); no *P. complanata* were found in other months, so the non-gravid period is unknown. No males were found, implying that *P. complanata* may be hermaphroditic. Pekkarinen (1993) found three hermaphrodites among 180 mussels in Finland, showing that hermaphroditism is present but uncommon in the Finnish population.

This study investigates the reproductive biology of two British populations of *P. complanata*, in the Great Ouse catchment and the Waveney and Yare catchment in East Anglia (Fig. 2). Both catchments contain short stretches with very high densities of *P. complanata* (McIvor, 1999; Müller, 1999). The gravidity, fecundity, sex ratio and possibility of hermaphroditism were studied to determine whether these populations are reproductively active, and to provide detailed information about their reproductive biology to inform future management plans aimed at this species' conservation.

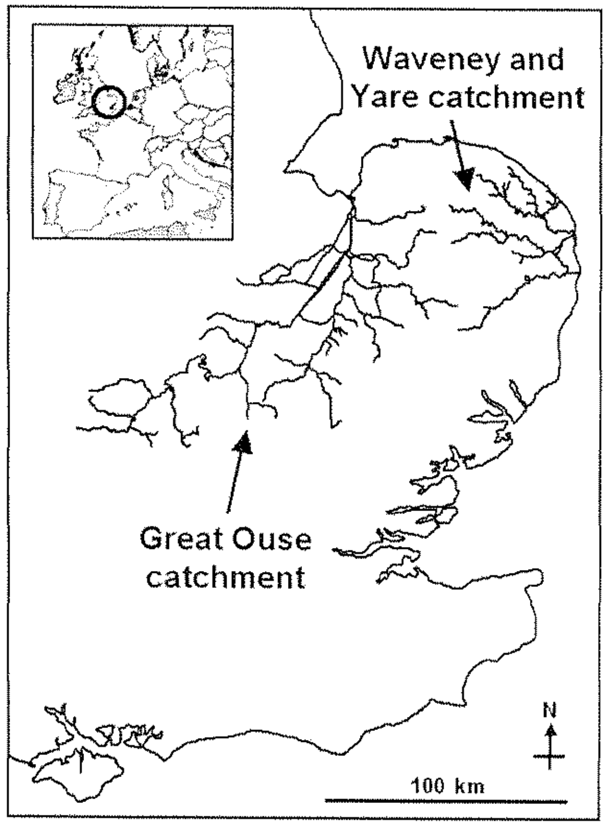

Figure 2. Map of southeast England showing the Great Ouse catchment and the Waveney and Yare catchment.

MATERIAL AND METHODS

Specimens of *P. complanata* were collected by hand every month between January and November 1999 from a range of locations within the Great Ouse catchment and the Waveney and Yare catchment in East Anglia, UK (Fig. 2). Mussels were collected from different locations each month to avoid collecting the same specimens twice.

The maximum lengths of all *P. complanata* were measured with vernier callipers. The valves of mussels were gently prised open by inserting the tips of the thumbs into the gape between the valves and pulling the valves a few millimetres apart (the permanent valve gape of *P. complanata* makes this possible). The demibranchs could then be examined *in vivo* to identify males, non-gravid females and gravid females. In males, there is no swelling of either the outer or inner demibranchs, which are both thin and flexible when lifted up with a blunt needle; in non-gravid females, the outer demibranchs are thicker than the inner demibranchs, and they are sturdier and more rigid; and in gravid females, the outer demibranchs are highly swollen along all or part of their length (following Bloomer, 1934; Heard, 1975; Hüby, 1988; Aldridge, personal observation).

Samples were taken from inside the outer demibranchs of gravid females using a syringe with a 600-μm bore needle to discover whether eggs, immature or mature glochidia were present. The samples were examined under a dissecting microscope, and mature glochidia snapped shut when a saturated salt

Table 1. The source catchment and lengths of the 14 mussels whose glochidia were counted (shown in bold) which form a sub-group of the 24 dissected mussels; these mussels were collected between February and April 1999.

Catchment	Sex	No.	Lengths (mm)
Great Ouse	Female	5	58, 59, **62**, **63**, **69**
	Male	1	54
Waveney/Yare	Female	11	**48, 55, 62, 62, 64, 64, 69, 76, 80, 80, 80**
	Male	7	32, 37, 43, 48, 63, 81, 85

solution was added (Weaver, Pardue & Neves, 1991; Watters & O'Dee, 1998; Fisher & Dimock, 2000).

The demibranchs from 14 fully gravid females (Table 1) were dissected out, cut open and shaken violently in water to flush out all glochidia from between the gill septae. Sub-samples from an agitated suspension of the glochidia were taken and the number of glochidia counted.

Gonadal tissue samples from the upper central portion of the foot tissue were taken from 24 mussels (Table 1), and these were passed through an alcohol series and into xylene and wax for sectioning with a microtome. Five 6-μm thick sections were cut at 200–300 μm intervals throughout the gonad; Loosanoff (1937), Heard (1975) and Grande, Araujo & Ramos (2001) emphasize the importance of looking at more than one region of gonad tissue when in search of hermaphroditic tissue. The sections were stained with haematoxylin and eosin. Spermatocytes and oocytes were identified using descriptions given by Dinamani (1974) and Peredo & Parada (1984).

RESULTS

The gonadal sex of 23 of the 24 dissected mussels matched the sex as determined by examination of the gills; in the one remaining mussel, no sex could be determined from the gills. This confirms the reliability of sexing mussels using the comparative thickness of the outer and inner demibranchs.

The sex ratio of mussels

Altogether 243 females and 112 males were found, giving a combined sex ratio of 2.5 females:1 male, which is significantly different from a 1:1 ratio ($\chi^2 = 60.8$, df = 1, $P < 0.001$, using the Yates correction for continuity; Zar, 1996). The sex ratio was not significantly different between the two catchments: 132 females and 63 males were found in the Ouse catchment, compared with 111 females and 49 males from the Waveney and Yare catchment ($\chi^2 = 0.115$, df = 1, $P = 0.734$). It was shown by t-tests that males were significantly longer than females in both catchments: Ouse catchment: $t = 7.09$, df = 93, $P < 0.001$; Waveney and Yare catchment: $t = 3.13$, df = 62, $P < 0.005$ (Fig. 3). *Pseudanodonta complanata* from the Waveney and Yare catchment were also significantly longer than those from the Ouse catchment: $t = 8.71$, df = 334, $P < 0.001$.

Gravidity of mussels

The smallest gravid mussels were 43 mm in length (Ouse catchment) and 49 mm in length (Waveney and Yare catchment). Smaller non-gravid mussels were found in both catchments as shown in Figure 3.

Mussels were gravid with mature glochidia from September through to April (Fig. 4). Following a short non-gravid interval in May, mussels started to brood eggs in their outer demibranchs in June and July. By August, these eggs had developed into glochidia, although the glochidia were only in the early stages of formation: they did not possess hooks and were white in colour. By September mature glochidia with well-formed light-brown hooks were present.

Glochidial release must have occurred in late April/early May, although this was not observed directly. It is probable that glochidia were not all released at the same time, because in April the demibranchs of 10 of the 22 gravid females were swollen for only part of their length; at other times of the year the demibranchs were swollen along most of their length.

Fecundity

Individuals contained between 5,000 and 50,000 glochidia in their outer demibranchs. The number of glochidia (n_{gloch}) increased with the length of the mussel (L, mm) as follows:

$$n_{gloch} = 3.52\,L^{2.1}$$

(linear regression on natural logarithm of data: $F_{(1,12)} = 11.4$; $P < 0.01$; $R^2 = 0.486$; Fig. 5).

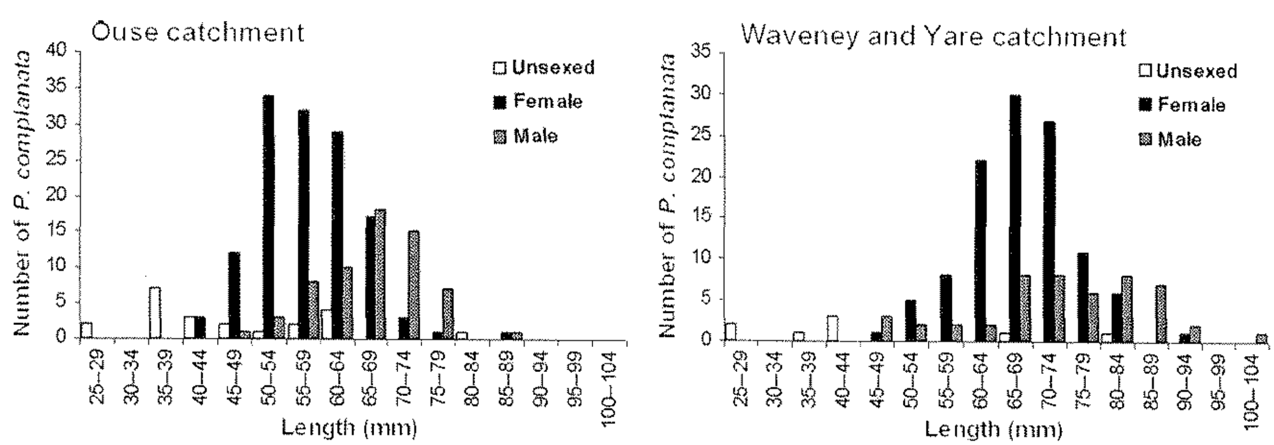

Figure 3. The size frequency distribution of male and female *Pseudanodonta complanata* in the Ouse catchment and the Waveney and Yare catchment, also showing the few mussels whose sex could not be determined by visual examination of the gills (mostly the smaller mussels).

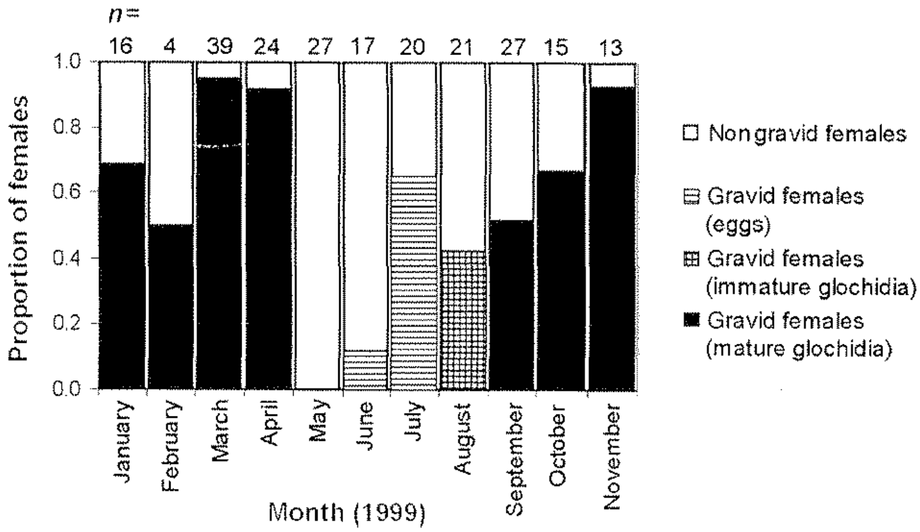

Figure 4. The proportion of *Pseudanodonta complanata* that was gravid each month, showing whether eggs, immature glochidia or mature glochidia were present. The number of mussels examined each month is given above each column. Data from the Great Ouse, the Waveney and the Yare were pooled as it was not possible to visit all catchments in all months.

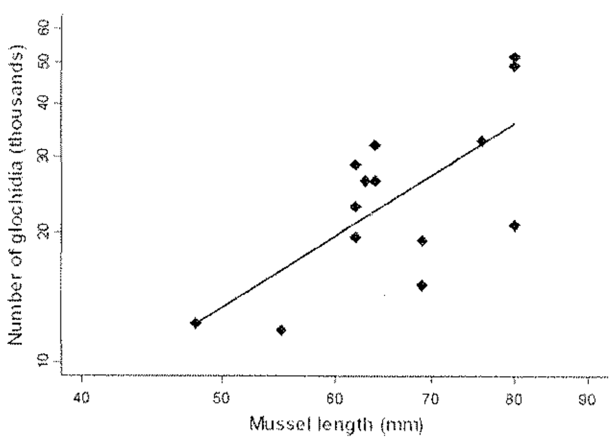

Figure 5. The relationship between the length of a female mussel and the number of glochidia being brooded in the outer demibranchs. The line shows a linear regression through the data (see text).

The gonadal sex of mussels

In the 24 dissected mussels, either spermatocytes or oocytes were visible in all gonadal sections (8 males, 16 females); no mussels contained both spermatocytes and oocytes.

Oocytes were recognized by their large size (between 50 and 80 μm diameter), large nucleus and granular cytoplasm (Fig. 6A). Spermatocytes were much smaller (4 μm diameter) and darkly stained throughout; large numbers were densely packed within the follicles (Fig. 6B). In some sections, spermatozoa were visible near the centre of the follicles; these were bullet shaped, long and thin with one end flattened and the other end rounded (3 μm long and 1 μm wide); occasionally their tails were also visible. Large multinucleated sperm morulae (up to 10 μm across) were also visible in male sections.

DISCUSSION

In the catchments studied, *Pseudanodonta complanata* was found to be reproductively active, producing viable glochidia. *Pseudanodonta complanata* is probably a generalist in its use of host fish species, as it encysts on a number of fish species, including the ruffe, *Gymnocephalus cernua* (Berrie & Boize, 1985), perch (*Perca fluviatilis*), zander (*Stizostedion lucioperca*), three and nine-spined sticklebacks (*Gasterosteus aculeatus* and *Pungitius pungitius*) and brown trout (*Salmo trutta fario*) (Hüby, 1988). Many of these fish species are present in the Ouse, Waveney and Yare catchments (Environment Agency, personal communications), and therefore *P. complanata* should be able to reproduce. The presence of smaller individuals (<30 mm in length in both catchments, Fig. 3) is confirmation that recruitment is occurring (specimens smaller than this are rarely found by hand-sampling). Therefore, these populations are considered important populations for conservation.

Sex ratio and hermaphroditism

A female-biased sex ratio similar to that seen here has been reported from a number of other freshwater bivalve species (Heard, 1975; Bauer, 1987; Byrne, 1998; Garner, Haggerty & Modlin, 1999). No hermaphrodites were found, suggesting that *P. complanata* is either wholly or predominantly dioecious in these catchments. Pekkarinen, (1993) found approximately 2% hermaphrodites in Finnish waterways, and similar levels could exist in British populations. Weisensee (1916) suggests that a skewed sex ratio could be a phase in the development of hermaphroditism in anodontine species, many of which are known to demonstrate great plasticity in their life history strategies (Heard, 1975).

Male *P. complanata* were generally longer than females. Similarly, Brander (1954) noted that in Finnish populations, females were under-represented in the larger size classes, and that the largest female was smaller than the largest male. A possible explanation for this is that females resorb their shells to provide calcium carbonate for juvenile shell formation; shell resorption (or negative growth) is seen in several freshwater bivalve species, including *Anodonta grandis grandis* and *Lampsilis radiata siliquoidea* (Downing & Downing, 1993), the zebra mussel *Dreissena polymorpha* (Hincks & Mackie, 1997), and *Anodonta anatina* and *Unio pictorum* (McIvor, 2004).

The sex determined from the gills matched gonadal sex in 23 out of 24 individuals, allowing the reliable estimation of sex

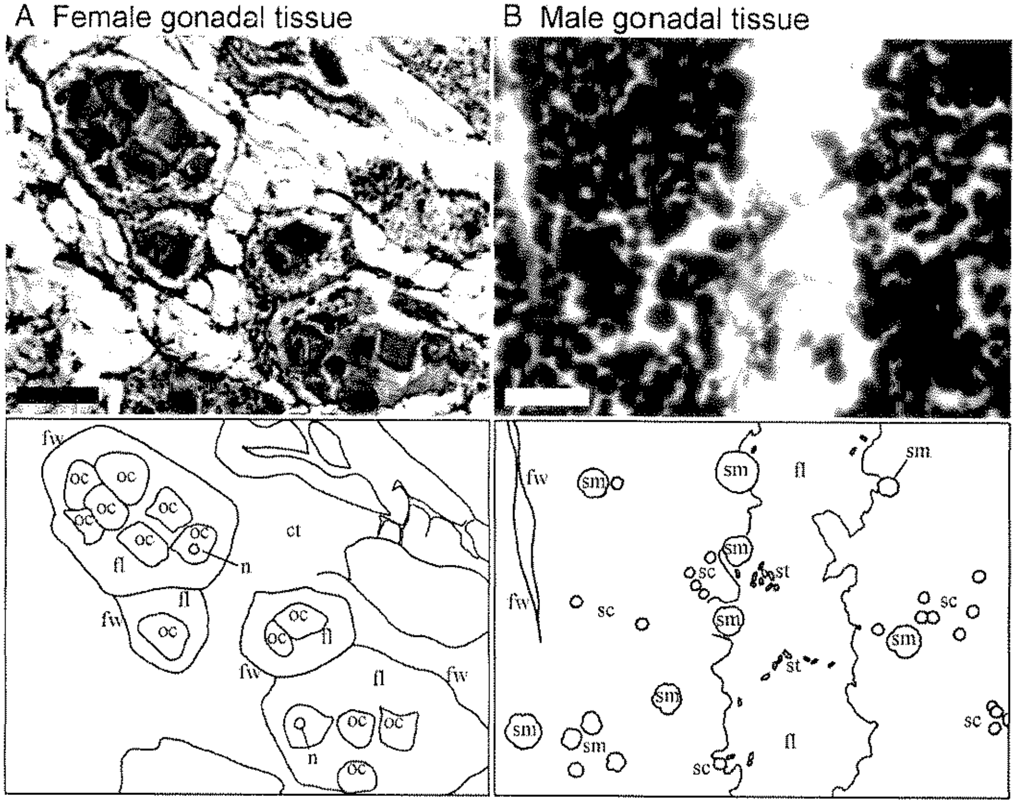

Figure 6. Gonadal tissue. **A.** Female gonadal tissue, showing oocytes (oc), nuclei (n), follicle lumen (fl), follicle wall (fw) and connective tissue (ct). Scale bar = 120 μm. Most follicles were empty or contained only a few oocytes, because the specimens were collected between February and April when the previous year's oocytes had already been released into the gills and the current year's oocytes had not yet started forming. **B.** Male gonadal tissue, showing spermatocytes (sc), spermatozoa (st), sperm morulae (sm), follicle lumen (fl), and follicle wall (fw). Scale bar = 25 μm. Photographs: S. Müller.

without the need to sacrifice mussels. This technique is particularly easy with *P. complanata* because of the long period of gravidity and the permanent valve gape, allowing the mussel valves to be prised open easily without the need for tongs and without damaging the mussel's shell.

Age at maturity

The smallest gravid females were 43 and 49 mm in length in the Ouse and Waveney and Yare catchments, respectively; these lengths correspond to an age of approximately 4 years old (estimated from catchment-specific length:age graphs produced by McIvor (1999) and Müller (1999), where age was measured by counting the growth rings on mussel shells). This falls within the normal range for age at maturity of unionids: Haag & Staton (2003) found that *Elliptio arca* and *Lampsilis ornata* matured by the age of two, while *Quadrula asperata* and *Q. pustulosa* reached maturity between the ages of three and nine.

The timing of gravidity

The non-gravid interval of *P. complanata* is in May in these catchments, which is similar to that seen in Finnish populations (in June, Pekkarinen, 1993), and in between the non-gravid periods of Austrian populations (March–May, Schierholz, 1889) and Russian populations (July–August, Zhadin, 1952). This pattern suggests that glochidial release occurs earlier in the year in warmer regions. This is consistent with the findings of Hüby (1988), who showed that glochidial release by *P. complanata* could be induced at 20°C with the addition of fish but not at 12°C, and Bloomer (1943), who recorded that low temperatures delayed or inhibited glochidial release in *Anodonta cygnea*. Hastie & Young (2003) document both inter-annual and between-catchment variation in the timing of egg transfer to the gills and glochidial release in Scottish populations of *Margaritifera margaritifera*, which they also attribute to variations in water temperature.

Fecundity

In this study between 5,000 and 50,000 glochidia were found in gravid females, and the number of glochidia increased exponentially with mussel length. Hüby (1988) found smaller numbers (between 8,000 and 16,000) in *P. complanata* from Germany, using mussels of a similar size. Both Hüby's data and the numbers of glochidia seen in this study are low relative to numbers seen in other European species: between 100,000 and 300,000 glochidia in *Unio* spp. (Maaβ, 1987; Piechocki, 1999) and 16,000,000 glochidia in *M. margaritifera* (Young & Williams, 1984). However, some North American species show similar small numbers of glochidia; for example, *Fusconaia cerina*, *Quadrula asperata* and *Q. pustuslosa* were found to produce <60,000 glochidia by Haag & Staton (2003).

Implications for conservation: recommendations for river management

Ideally, channel management of river stretches containing important *P. complanata* populations should be kept to a minimum, as any form of disturbance is likely to affect the mussels. In the UK, common forms of channel management used to maintain flows and thus prevent flooding include dredging (to prevent the build up of sediment) and weed-cutting (to remove excess growth of macrophytes). Both methods remove mussels from the river channel (McIvor, 1999; Aldridge, 2000, 2004); in an extreme case, a single dredging event in the River Brue in Somerset removed more than 20% of a *P. complanata* population (Müller, 1999). The requirement for such management should be carefully considered, and only undertaken if absolutely necessary and if the benefits of such management can be shown to outweigh the potential disturbance to the *P. complanata* population. If such work is considered essential, the following recommendations may reduce the negative impacts on populations.

Forms of river management which preferentially remove larger mussels, such as the Bradshaw bucket (a mechanical weed-cutting bucket mounted on a bank-side excavator) should be avoided, substituting practices which are not size-selective, such as weed-cutting either using a boat or by hand (Aldridge, 2000). Removing the largest mussels will not only remove a disproportionate number of males (shown here to be larger), but will also remove the most fecund females, which could result in a dramatic reduction in the numbers of glochidia produced. Although weed-cutting by boat or by hand is more expensive, dense populations of *P. complanata* are often highly localized along short stretches of river (Müller, 1999; McIvor, 1999; D.C. Aldridge, unpubl.), so the cost need not be prohibitive.

As mussels may release larvae prematurely when stressed, e.g. by physical disturbance, exposure to high turbidity or low oxygen concentrations (Aldridge & McIvor, 2003), it is recommended that work be carried out during the non-gravid period of mussels (i.e. May), to prevent causing the premature release of larvae which could result in a large proportion of mussels foregoing reproduction for that year. However, the very short non-gravid period may be insufficient to complete management works, particularly longer term construction operations (e.g. bridge building). In such cases, it is recommended that works be carried out within one reproductive year of mussels (i.e. approximately June–May) so that only one reproductive cycle is affected.

The frequency of channel management should also be considered. If large numbers of mussels are removed during management operations (as in dredging), and if the interval between dredging is shorter than the time it takes a mussel to become reproductively mature, then whole populations of mussels may be lost. The youngest reproductively active individuals of *P. complanata* were approximately 4 years old; therefore, it is recommended that a dredging frequency of not more than once per 6 years is carried out, and longer intervals should be preferred.

Finally, the temporary translocation of mussels should be considered in the case where an isolated population will be impacted by a management procedure that is likely to remove most of the mussels. If mussels are carefully handled and well maintained, either in holding tanks with a suitable algal diet (Gatenby *et al.*, 2000) or in net bags suspended in the source river (McIvor, 2004), high survival rates can be achieved. Mussel relocation has become a common procedure for important unionid populations in North America (Cope & Waller, 1995).

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