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First observations of spawning nests in the pouched lamprey *Geotria australis*

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1 **First observations of spawning nests in the pouched lamprey (*Geotria australis*)**

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17 **Running title:** Observations of *Geotria australis* spawning nests

18

19 **Abstract**

20 The pouched lamprey, *Geotria australis*, one of four Southern Hemisphere lamprey species,
21 is New Zealand's only freshwater representative of the agnathans. In contrast to Northern
22 Hemisphere lampreys, the reproductive ecology of Southern Hemisphere lampreys is poorly
23 understood with no documented nest sites or spawning behaviours. In the present study we
24 utilised PIT tags to track migratory adult *G. australis* to locate spawning sites. Across two
25 years, six cryptic nest sites were discovered, each containing eggs and a single male and

female lamprey. The post-spawning pairs were all located underneath large boulders with the eggs forming a coagulated cluster that adhered to the underside of the boulder. Both the male and female lamprey survived spawning for over 105 days. The observed spawning nests highlight a substantial divergence in the reproductive behaviour between Northern and Southern Hemisphere lampreys, as the present observations of *G. australis* are the first that support clustered cryptic egg deposition and an extended time to mortality post-spawning. This study offers the first insights to the long-standing mystery of Southern Hemisphere lamprey reproduction.

Key words: lamprey, spawning, *G. australis*, reproduction, PIT

Introduction

Lampreys can be regarded as “living fossils” with a lineage that diverged from a common ancestor with jawed vertebrates some 500 million years ago (Janvier 2007). Extant lampreys have an antitropical distribution, with the Northern Hemisphere Petromyzontidae family containing 37 lamprey species, and the four Southern Hemisphere lamprey species assigned to the families Mordaciidae (three species), and Geotriidae (one species) (Potter et al. 2015). The pouched lamprey, *Geotria australis*, Gray 1851, is the sole Geotriidae species, and New Zealand's only freshwater representative of the agnathans (jawless vertebrate). *G. australis* has a wide southern temperate distribution ranging from Western Australia to Argentina (McDowall 1990). Although electrophoretic analysis has previously indicated genetic homogeneity of stocks in Australia and New Zealand (Johnston et al. 1987), Neira et al. (1988) found some obvious morphological differences between the ammocoetes of *G. australis* from Australasia (Australia and New Zealand), Argentina, and Chile. Therefore,

1 further investigations are necessary to ascertain whether *Geotria* comprises more than a
2 single species.

3 In New Zealand, migratory adult *G. australis* (also known as kanakana or piharau) form an
4 important cultural fishery, and are considered a taonga (treasured) species by indigenous
5 Māori (McDowall 2011). Although empirical data are lacking, there is anecdotal evidence of
6 a historic decline in the abundance of lamprey through continued loss of suitable freshwater
7 habitat (McDowall 2011). Presently, *G. australis* are classified as “Threatened - Nationally
8 Vulnerable”, in the New Zealand Threat Classification (Goodman et al. 2014). However,
9 current conservation efforts are hampered by the limited knowledge of their ecology in the
10 freshwater environment, particularly with respect to reproduction.

11 *G. australis* has an anadromous life history with spawning and larval development taking
12 place in fresh water (McDowall 1990). After 3 to 4 years, the larvae metamorphose into
13 macropthalmia, which migrate to the ocean and feed parasitically on fish and marine
14 mammals (Kelso and Todd 1993). Adults return to fresh water between late autumn and early
15 spring, where they spend 14-16 months maturing before spawning and are presumed to die
16 shortly afterwards (Kelso and Glova 1993; Jellyman et al. 2002; Glova 1995). In contrast to
17 that described for Northern Hemisphere lamprey species, the reproductive ecology of *G.*
18 *australis*, and the three other Southern Hemisphere lamprey species is poorly understood with
19 no documented nest sites or spawning behaviours (Johnson et al. 2015).

20 In this study we captured, tagged and monitored migrating *G. australis* en route to spawning
21 locations throughout the expected 15 month duration of the spawning migration. Our
22 primary research objective was to locate spawning-phase lamprey and document physical
23 characteristics of their nest sites with a focus on detecting differences from habitats selected
24 by pre-spawning individuals. Our secondary objectives were to document behaviours and

morphological sexual characteristics of adult *G. australis*, and monitor the development of eggs from the time of deposition through to hatching and larval emergence. This paper describes the first observations of a Southern Hemisphere lamprey species nesting sites and nesting behaviour.

Materials and Methods

Study site

Geotria australis were monitored in the Okuti River catchment, Banks Peninsula, South Island, New Zealand (175°15'E, 37°64'S, Fig. 1) as previous studies by Kelso and Todd (1993) and Jellyman et al. (2002) had identified reliable recruitment of adult fish occurs in the catchment. The Okuti River enters Lake Wairewa, a coastal lake that is separated from the ocean by a gravel bar. The separation of Lake Wairewa from the ocean occurs naturally due to waves washing gravel into, and blocking, the mouth of the lake. The bar is mechanically opened at predetermined lake levels to prevent flooding of the surrounding land. In general, after opening, the bar will naturally close again within 48 hours. Entry of adult lamprey was therefore restricted to short periods when the lake was open, which allowed the timing of the migration runs to be reliably predicted. In the study stream, adult lamprey migration season occurs predominantly from July through September each year. Therefore, lake openings over this period were targeted. Across the study, the lake opened once in early August 2012, and once in late July 2014. In 2013, the lake did not open between July and September, therefore, no lamprey were able to be captured that year.

An unnamed tributary, hereafter referred to as Kinloch Stream, was the primary study site (Fig. 1). Kinloch Stream averages 2 m wide and the majority of the stream is <0.4 m deep, with a mean annual flow of 0.12 m³ s⁻¹. Instream habitat is approximately 40:40:20 riffle, run and pool habitats containing a predominantly boulder and cobble substrate. The riparian zone

1 is dominated by rank grasses and exposed substrate, with canopy cover present along both
2 banks for the majority of the stream length.

3 Water levels in Kinloch Stream were monitored continuously (15 minute intervals) using
4 Odyssey™ (Dataflow Systems, Christchurch, New Zealand) capacitance water level loggers
5 and HOBO water temperature loggers (Onset Computer Corporation, Bourne, Massachusetts,
6 USA) monitored stream temperature at 15 minute intervals. A malfunction of the water
7 temperature logger in October and early November 2015 prevented the water temperature
8 from being recorded at the time of spawning.

9 *Tagging and tracking of fish*

10 In 2012 and 2014, migratory adult lamprey were captured from the Okuti River and Kinloch
11 Stream (Fig. 1) during their upriver migration. To maximize catch efficacy of lamprey, a
12 combination of methods was utilised following Kelso and Glova (1993) and Jellyman et al.
13 (2002). Two double-wing fyke nets (capturing 51% of lamprey) were placed in the lower
14 reaches of the Okuti River (approximately 8.8 km inland from the coast; Fig. 1), with
15 backpack electric-fishing (NIWA Kainga EFM300; capturing 27% of lamprey) undertaken
16 from the entrance of Kinloch Stream up to the road culvert (Fig. 1). In addition, two custom
17 built conduit pipe traps were placed at a small concrete weir immediately below the road
18 culvert in Kinloch Stream, which captured 22% of lamprey (Fig. 1). Overall, 141 adult
19 lamprey were captured between 7 August and 19 September 2012, and 55 adult lamprey were
20 captured between 1 August and 19 November 2014.

21 Immediately after capture, all lamprey were sedated with AQUI-S® and using a needle and
22 syringe, 12 mm HDX Passive Integrated Transponder (PIT) tags (Texas Instruments, Austin,
23 Texas, USA) were inserted into the body cavity along the midline approximately half way
24 along their longitudinal axis. Although active tags (radio and acoustic) are more effective for

1 tracking fish movements, the limited space in the visceral cavity of adult *G. australis*, which
2 can be as little as 2 mm in width, necessitated the use of PIT tags. Jellyman et al. (2002)
3 attempted to track adult *G. australis* to spawning grounds using externally mounted radio tags
4 but retention was problematic and all 65 fish lost their tags within 56 days.

5 Following implantation, lamprey were left in a bucket with clean stream water until normal
6 ventilation returned before being released. In 2013, to enable the present spawning site study
7 and a separate stream selection investigation to be undertaken, lamprey were released in both
8 the Okuti River and Kinloch Stream. In total, 115 lamprey were released into the Okuti River,
9 approximately 35 m downstream of the confluence with Kinloch Stream, and 26 lamprey
10 were released directly into Kinloch Stream 10 m upstream of the road culvert (Fig. 1). The
11 length (T_L mm) and mass (g) of each lamprey was also recorded prior to release. In 2014, all
12 55 lamprey captured and tagged were released into Kinloch Stream 10 m upstream of the
13 road culvert.

14 Tagged lamprey were monitored with a portable HDX PIT backpack reader (Oregon RFID,
15 Portland, Oregon, USA) using a circular antenna wand (0.5 m diameter). Instream trials using
16 planted tags showed that within boulder substrate, the read range of the 12 mm HDX tag
17 (parallel to the antenna) was 29 cm. Because the larger Okuti River was dominated by large
18 boulders and the detection efficiency of the 12 mm HDX tag was found to be less than 5%,
19 monitoring of lamprey to locate spawning and nesting sites was only carried out in Kinloch
20 Stream. For this reason, tagged lamprey were only released into Kinloch Stream in 2014.

21 During the migration and spawning season (September to December 2012 through 2015)
22 between two and four stream walks with the portable PIT reader were undertaken each
23 month. A Garmin GPS was used to record the position of all tagged lamprey detected. To
24 examine the movements of lamprey while maturing within freshwater, between January and

1 August 2013 and 2015, tagged lamprey were monitored at approximately two monthly
2 intervals.

3 In addition, two fixed antennae installed near the confluence of Kinloch Stream and the Okuti
4 River allowed the number of lamprey migrating into and out of Kinloch Stream to be
5 monitored.

6 *Nesting sites*

7 Between 21 - 25 October 2013 and 2 - 6 November 2015, intensive searches for spawning
8 lamprey were undertaken. Monitoring with the portable PIT reader had determined that
9 lamprey were residing underneath large boulders within the stream during daylight hours. As
10 daytime searches of cobble and gravel habitats characteristically used for spawning by
11 Petromyzontidae lampreys had failed to locate nests in previous years (authors' unpublished
12 data), spawning surveys were carried out on three nights in November 2012, and one night in
13 October 2013. Surveys focused on approximately 200 m of suitable habitat in the lower
14 reaches of Kinloch Stream where tagged lamprey were detected during the day. As not a
15 single lamprey was observed across all three nights, in October 2013, boulders were
16 overturned when tags were detected to examine the maturity of individual males and females.
17 Lamprey were categorised as being either migrant (fresh run adults still blue in colour),
18 maturing (adult lamprey maturing in freshwater that are brown in colour with the male gular
19 pouch developing and the female exhibiting no morphological changes associated with sexual
20 maturity), pre-spawning (male and female pair located underneath a boulder with the male
21 exhibiting the gular pouch and the female heavily gravid) or post-spawning (male and female
22 pair located beneath a boulder with the male exhibiting the gular pouch, the female emaciated
23 and an egg clump evident). After locating the first nest site, any large boulder deemed

1 suitable for spawning under was overturned in an effort to locate nests of untagged fish or
2 fish that were not being detected by the portable PIT system.

3 In 2015, a less intrusive approach was undertaken to enable observations of the lamprey in
4 their nests. Boulders with tagged lamprey detected beneath and similar boulders deemed
5 suitable for spawning had a small hole excavated and an 8 mm diameter inspection camera
6 (Bullant G5000) was used to determine if lamprey and eggs were present. Of the three nests
7 found, two were monitored with the inspection camera at weekly intervals during larval
8 development, and twice a week when the larvae were hatching. After the larvae had left the
9 nests, monitoring was undertaken at approximately fortnightly intervals to document the time
10 to mortality of the spent lamprey. On each occasion, approximately 15 min of video footage
11 was recorded from each nest site. After each inspection, the hole was infilled to prevent
12 longfin eels (*Anguilla dieffenbachii*) preying on the nest site.

13 In both years, when a nest was located, habitat measurements were carried out. The habitat
14 lamprey were nesting in was visually categorised as a backwater (still water environments
15 with no inflow at the head), pool (deeper, slow-flowing water with an inflow and outflow),
16 run (moderate water depth with reasonably swift waters), or a riffle (shallow swift waters
17 with coarse cobble sediments). Habitat dimensions including maximum water depth, instream
18 water velocity (using a Marsh-McBirney FloMate™ 2000), and wetted width were recorded.
19 Three representative depths, velocities and wetted widths that covered the range present were
20 also recorded to calculate average water depth, water velocity and average habitat width. A
21 visual assessment of riparian cover was undertaken and expressed as percentages of exposed
22 substrate, native vegetation, exotic vegetation, grasses, scrub, and flax. The dimensions of the
23 boulder where each nest was found were recorded, along with the other types of cover present
24 in the habitat. For this purpose, cover was defined as boulder, aquatic macrophytes, root

1 mass, small debris (i.e., twigs and leaves), undercut bank, log or debris dam. Substrate
2 composition was visually categorised into the percentage area occupied by bedrock, boulder,
3 cobble, coarse gravel, fine gravel, sand, and mud, using the modified Wentworth particle size
4 scale (Bovee and Milhous 1978). Substrate estimates were converted to a single index by
5 summing weighted substrate percentages (Jowett and Richardson 1990; Jowett et al. 1991),
6 where substrate index = 0.08 bedrock% + 0.07 boulder% + 0.06 cobble% + 0.05 coarse
7 gravel% + 0.04 fine gravel% + 0.03 sand% + 0.02 mud% + 0.01 vegetation%. Accordingly,
8 the substrate index ranges from 1 (vegetation) to 8 (bedrock).

9 To compare habitat used during migration to that utilised during nesting, on the 4th October
10 and 14th November 2012, and the 21st March 2013, habitat assessments were also carried out
11 at 18 sites where maturing lamprey were located after entry into Kinloch Stream. The habitat
12 assessments for these maturing fish and also for nesting fish were timed to coincide with base
13 flow conditions. Mann-Whitney U tests were performed to determine if habitat utilisation
14 (boulder size, water depth, water velocity, substrate index and wetted width) was
15 significantly different between maturing and nesting lamprey.

16 *Egg development*

17 Samples of eggs from two nest sites were taken back to the NIWA Christchurch laboratory to
18 confirm the eggs were in fact from *G. australis*. Genetic testing was unnecessary as *G.*
19 *australis* is the sole lamprey species in New Zealand, and hatching larvae would therefore
20 provide conclusive identification. To reduce development time and ensure visits to the field
21 sites did not miss key events, eggs were reared in warmer conditions than that of the study
22 stream (daily mean water temperature rose from 11.1°C at spawning to 13.3°C at hatching) in
23 an 18°C temperature controlled room. Development of the larvae was monitored through to
24 hatching.

The one pre-spawning female that was located was euthanized and a fecundity estimate was undertaken. The eggs were removed from the ovary and weighed (wet weight measured in grams) before removing and weighing 8 subsamples of eggs. Each sub-sample was fixed in about 1 mL of Gilson's fluid (Bagenal and Braum 1978) to break down the ovarian tissue and liberate the eggs. Each ovary sub-sample was then spread evenly in a glass petri dish and a scan taken using a HP Scanjet G4010. The total number of eggs in each sub-sample was then counted using a pre-calibrated macro run on Image J (version 1.37) analysis software with egg diameter also measured. The sub-sample counts were used to calculate the average egg weight so that a total fecundity estimate could be made.

Results

Nesting sites

In 2012 and 2014, 40 and 37 of the tagged lamprey, respectively, had entered or remained after release within Kinloch Stream. Of these fish, five were able to be monitored through the 15 month maturation period in freshwater to post-spawning in late October 2013 (three males and two females), and two fish (male and female) in November 2015. Spawning searches during November 2015 also located one additional nest site that contained untagged lamprey. In 2013, when the boulders were overturned, male lamprey immediately attempted to flee, whereas female fish were slower moving and easy to capture by hand. Egg deposition had already occurred at all nest sites when discovered. In 2013, at the estimated time of spawning, the average daily stream temperature was 11.1°C (daily max 13.0°C, daily min 9.7°C).

Across the two years, six nest sites were discovered (Fig. 1), each containing eggs and a single male and female lamprey. The lamprey pairs and eggs were all located and completely concealed underneath large boulders. The circumference of each boulder was in contact with the stream bed so that there were no openings that enabled the 8 mm diameter camera head

1 and tube access under the boulder. As such, sediment was excavated to enable entry of the
2 camera and observations of the nest.

3 Two nests were beneath boulders in backwaters adjacent to riffle habitat, and four nests
4 amidst boulder clusters within shallow riffles (Fig. 2). The substrate indices at the nest sites
5 were indicative of the large boulder habitat utilised and the shallow water depths recorded
6 were representative of the riffle habitat present in Kinloch Stream (Table 1).

7 In comparison to the habitat utilised by nesting lamprey, maturing lamprey were found
8 predominantly in run (50%) and riffle (37%) habitats during the first seven months after
9 entering Kinloch Stream. The key difference recorded between nesting lamprey and maturing
10 adults, was the size of the boulder chosen for cover. The nest sites were all located under
11 significantly larger boulders than those maturing lamprey resided beneath ($P < 0.047$, Mann-
12 Whitney U tests, Table 1).

13 *Behaviour and morphology of post-spawning fish*

14 Of the two post-spawning lamprey pairs visually monitored in 2015, both the male and
15 female remained alive at each nest for over 105 days (see online supplementary material Fig.
16 S1¹). The last observation of both pairs was the 17 February 2016. By 1 March 2016, no
17 lamprey were observed at either nest site, and a longfin eel was present in one nest.

18 Across the monitoring period, neither sex appeared to enter a state of quiescence, instead they
19 remained active within the nest. The two female lamprey were not observed to interact with
20 the developing eggs, nor show an interest in the camera. In contrast, the males were very
21 active and would approach the camera and egg mass once observations began. Both male
22 lamprey were aggressive towards the camera and frequently lunged at the lens with one male

¹ Supplementary data are available with the article through the journal Web site

observed to wrap himself around the developing egg mass after the camera intrusion (Fig. 3). The gular pouch of the male lamprey was often observed to massage or move the eggs around as the males moved past and around the egg mass. During hatching the male was also observed to vigorously rub his gular pouch and ventral surface across the eggs in kneading and rocking motions using both dorsoventral and lateral movements (Fig. S2¹). This behaviour was not observed prior to or once larvae had hatched.

Across both monitoring years, a morphological characteristic evident in all six sexually mature females but absent in male lamprey, was the presence of a raised dorsal ridge in front of the anterior dorsal fin (Fig. 4), visually similar to the 'rope' of the male sea lamprey (Hardisty and Potter 1971; Chung-Davidson et al. 2013).

Egg development

The eggs appeared as a coagulated cluster that adhered both to each other and to the underneath of the boulder (Fig. 2). The clusters were approximately 140 mm long by 110 mm wide with the egg mass varying in height between 3 mm and 26 mm across the clusters. The eggs were cream coloured, slightly ovoid in shape. Microscopic examination of the eggs after collection found they were in the early blastula stage with no animal pole differentiation evident (Reyes 2008). Based on the blastula occurring at one to four days post-spawning in the Pacific lamprey (*Entosphenus tridentatus*) when reared at 12 °C (Reyes 2008), it is likely that at all sites, the eggs were spawned within a few days of discovery. The fecundity calculated from one female was 56,100 eggs with a mean egg diameter of 1.18 mm (SE = 0.02 mm).

Egg development monitored in-situ at one nest site in 2013 and two nest sites in 2015, indicated that the larvae began hatching after approximately six weeks (3 December 2013 and 8 December 2015) and continued to hatch for four days (Fig. 5). At the time of hatching, the

1 average daily stream temperature had risen to 13.3°C in 2013 (daily max 16.5°C, daily min
2 10.1°C) and 12.9°C in 2015 (daily max 15.4°C, daily min 11.0°C).

3 After hatching, larvae were observed to form clusters within the nest, adhering to the
4 subsurface of the boulders and were not displaced even when disturbed by adult movements
5 (Fig. 5a). A cursory examination of larvae taken from a nest 10 days post-hatching found the
6 caudal region of each larva was adhesive and the larvae would readily attach to debris or any
7 solid surface they were exposed to (Fig. 6). Within a laboratory aquarium, larvae would
8 attach to small stones or debris rather than burrowing into the sediment. Larvae still remained
9 within the nests two weeks post-hatching on 23 December 2015 (Fig. 5b) with only a handful
10 of larvae present by 1 January 2016. From laboratory observations, at 15 days post-hatching,
11 the mean larval size was 7.2 mm (T_L , ± 0.30 mm S.E.).

12 Discussion

13 This is the first reported documentation of nesting sites for a Southern Hemisphere lamprey
14 species. Both the stream habitat utilised and nesting behaviour undertaken by *G. australis* is
15 considerably different to that characteristic of the Northern Hemisphere lamprey species.

16 Within the Petromyzontidae family, spawning behaviours are generally analogous among
17 species (Johnson et al. 2015). Lampreys create redd-like nests in riffle habitats, commonly
18 observed at the head of shallow riffles at transition areas between runs and riffles (Johnson et
19 al. 2015). Specific microhabitats vary among the species but in general a horseshoe shaped
20 depression is created in gravels and cobbles (Beamish 1980; Cochran and Lyons 2004; Stone
21 2006; Gunckel et al. 2009). Mating is primarily polygynandrous (multiple males mate with
22 multiple females) with the size of the aggregations reducing as body size of the species
23 increases (Johnson et al. 2015). The two larger lamprey species (>35 cm T_L), the sea lamprey
24 (*Petromyzon marinus*) and Pacific lamprey (*Entosphenus tridentatus*), have generally fewer

1 than five individuals per nest (Stone 2006; Gardner et al. 2012). Eggs are adhesive and attach
2 to the substrate but tend to scatter and do not remain as a single cluster (Silva et al. 2014;
3 Johnson et al. 2015). As *G. australis* is a large lamprey species (mean size 50-60 cm T_L),
4 spawning in pairs is not unexpected. Although the act of spawning was not witnessed, given
5 each lamprey pair was concealed beneath a large (and sometimes buried) boulder it is likely
6 that spawning involved just the two spent fish observed in each nest. However, in contrast to
7 Northern Hemisphere lamprey species, nest sites in *G. australis* have only been observed
8 underneath large boulders with eggs remaining as a coagulated cluster that adhere to the
9 underside of the boulder. Although sample sizes are small, the nesting habitat used by *G.*
10 *australis* remained consistent across two years (2013 and 2015), which suggests spawning
11 beneath boulders may be the preferred location for this species.

12 The utilisation of cover during spawning is less frequently observed in Petromyzontid
13 lamprey species. The chestnut lamprey (*Ichthyomyzon castaneus*), northern brook lamprey (*I.*
14 *fossor*), southern brook lamprey (*I. gagei*), western brook lamprey (*Lampetra richardsoni*),
15 Pacific lamprey (*Entosphenus tirdentalus*) and the European brook lamprey (*L. planeri*) have
16 all been reported to spawn beneath cover objects such as boulders, woody debris and
17 vegetation (Cochran and Gripentrog 1992; Gunckel et al. 2009; Johnson et al. 2015). The egg
18 deposition of chestnut, northern brook and southern brook lampreys was observed to be
19 partially concealed by the overhanging edge of an adjacent boulder or log, as well as in
20 interstices beneath boulders and rocks (Cochran and Gripentrog 1992). Gunckel et al. (2009)
21 found 86% of western brook lamprey nests were associated with cover such as rocky
22 substrates, wood and vegetation, with 43% of Pacific lamprey nests associated with cover in
23 the form of larger substrates, vegetation and wood. However, completely concealed cryptic
24 nests underneath large boulders, such as that observed in *G. australis*, has not been
25 commonly observed in Northern Hemisphere lamprey species. Cochran and Gripentrog

1 (1992) hypothesised that nesting beneath cover would occur more frequently in larger rivers
2 where swift currents may reduce spawning success in open riffle habitats, or as a means of
3 reducing predation.

4 The cryptic nesting habitats of *G. australis* are unlikely to relate to high water velocities
5 during mean or base flow conditions in the small shallow study stream, or many of the
6 tributaries *G. australis* inhabit within New Zealand. In addition, habitat availability is
7 unlikely to be the key factor in selection of cryptic nest sites as the microhabitats typically
8 utilised for spawning by Peteromyzontidae lampreys were not only present within the study
9 stream but also prevalent in many regions across New Zealand (Duncan and Woods 2013). In
10 particular, cobble-bedded braided rivers predominate in the South island and eastern North
11 Island of New Zealand. Spawning surveys have also previously been carried out in four other
12 Banks Peninsula streams that are known to be utilised by lamprey, but no nests were found in
13 reaches of open water (D. Jellyman, NIWA, pers. comm.). Further, fishery managers
14 throughout New Zealand frequently undertake spawning surveys for brown (*Salmo trutta*)
15 and rainbow trout (*Oncorhynchus mykiss*) during periods that overlap with lamprey
16 spawning, but there have been no reported observations of lamprey nests in such open water
17 habitats (D. Jellyman, NIWA, pers. comm.). Instead, we propose that reducing susceptibility
18 to predation is a key driver of spawning habitat selection in *G. australis*. Migratory adult *G.*
19 *australis* can penetrate well inland and to high elevations with records in New Zealand up to
20 507 m above sea level and 220 km inland (New Zealand Freshwater Fish Database,
21 <https://www.niwa.co.nz/our-services/online-services/freshwater-fish-database>). At high
22 elevations, *G. australis* would evade most natural predators except the endemic longfin eel
23 (*Anguilla dieffenbachii*), which generally constitutes the highest fish biomass of inland fish
24 populations, and is commonly associated with the boulder riffle habitats that adult *G.*
25 *australis* occupy. The longfin eel is an apex predator reaching sizes in excess of 1.3 m (T_L)

1 and 20 kg (McDowall 1990). Analyses of longfin eel stomach contents during spring have
2 found high numbers of fresh-run adult lamprey, with a large longfin eel reported to contain
3 12 adult lamprey (M. Bonnett, NIWA, pers. comm.). Similarly, in Australian waters, the
4 speckled longfin eel (*A. reinhardtii*) is another large (up to 1.6 m T_L and 14 kg) predator that
5 is widespread within sub-tropical and temperate regions (McDowall 1990). The diet of large
6 individuals is mainly comprised of fish (Beumer 1979), so it is likely that the speckled
7 longfin eel will also be an active predator of *G. australis* within inland Australian rivers.

8 A secondary advantage to spawning and nesting beneath large boulders is the reduction in
9 disturbance or displacement of the nest during flood flows. Most unregulated rivers and
10 streams are characterised by unpredictable flood regimes (Poff and Ward 1989), and in New
11 Zealand by their high flow variability (Poff et al. 2006). Consequently, it is not uncommon for
12 lotic species to utilise coarse substrates for protection from flood waters. Although
13 speculative, the shift to larger boulders observed between maturing and spawning adult
14 lamprey, may be a means of increasing protection of the nest from high discharge events.
15 Heggenes (1988) suggested that coarse substrates were essential in preventing displacement
16 of brown trout (*Salmo trutta*) during flood flows in a small stream and in analysing seven
17 New Zealand rivers before and after a large flood, Jowett and Richardson (1989) found there
18 was significant scouring of pools but little change of run and riffle habitats. McEwan and Joy
19 (2013) also found redfin bullies (*Gobiomorphus huttoni*) were displaced, or moved to areas
20 with larger substrates during flood waters in a small stream.

21 Results from the present study suggest that male *G. australis* may exhibit some form of
22 parental care or guarding of the eggs mass. In particular, the gular pouch may be used in
23 grooming of the eggs as it was observed to massage the eggs during development and was
24 vigorously rubbed against the egg mass during hatching. Several roles for the gular pouch of

1 *G. australis* have previously been proposed including for courtship or during the act of
2 spawning (Potter and Welsch 1997), for use in nest building (Maskell 1929) and as a nutrient
3 storage structure (Monette and Renaud 2005). The present study does not discount these
4 hypotheses, but provides support for the involvement of the gular pouch in egg grooming and
5 hatching. Currently, the behaviours of adult lamprey have only been observed at two nest
6 sites and therefore further investigations are necessary to confirm the role both male and
7 female *G. australis* undertake during egg development. Although the female lamprey were
8 not observed to interact with the developing eggs, visual observations were infrequent and
9 therefore could have missed key behaviours carried out by the female parents.

10 Parental care of nest sites is seen in many fish species, and aside from driving away
11 predators, generally consists of fanning and mouthing eggs to aid oxygenation and remove
12 metabolic wastes (Ghosh et al. 2012). As *G. australis* eggs develop underneath boulders
13 where water transfer will be reduced compared to open instream habitats, one of the drivers
14 behind adults remaining at the nest site may be to ventilate and increase oxygenation of the
15 developing eggs. In eleotrid bullies (*Gobiomorphus* spp), the male remains to guard the eggs,
16 excluding other fish from the site and occasionally fanning the nest to increase oxygenation
17 (McDowall 1990; McDowall and Eldon 1997). Ghosh et al. (2012) found that hatching
18 success in five species of clownfish (*Amphiprion sebae*, *A. clarkii*, *A. percula*, *A. ocellaris*
19 and *Premnas biaculeatus*) was positively correlated to the nest fanning duration and
20 frequency exhibited by the adult male.

21 A further difference documented between Petromyzontid lampreys and *G. australis* was the
22 time to mortality post-spawning. In the two nests monitored, both male and female *G.*
23 *australis* survived spawning for over 105 days. In comparison, most Petromyzontid lampreys
24 die within a month of spawning (Johnson et al. 2015), with only several accounts of lampreys

1 surviving for extended periods post-spawning. For example, Pletcher (1963) observed female
2 western brook lamprey die within a week of spawning but male lamprey would live between
3 1-2 months before death, and Korolyev and Reshetnikov (2008) found the European brook
4 lamprey would hide beneath stones after spawning and remain in a quiescent state for several
5 months before perishing.

6 Sexually mature female *G. australis* possessed a raised ridge of tissue in front of the anterior
7 dorsal fin, which resembled the 'rope' seen in mature male sea lamprey. At the final stage of
8 sexual maturation, adult male sea lamprey develop a rope-like thickening on their dorsal
9 ridge that extends from the posterior gill slit to the beginning of the anterior dorsal fin
10 (Chung-Davidson et al. 2013). Presently believed unique to male sea lamprey, the rope is a
11 thermogenic secondary sexual characteristic that produces heat in the presence of an ovulated
12 female, but not in the presence of other males (Chung-Davidson et al. 2013). It therefore
13 warrants further investigation to determine if indeed the rope observed in female *G. australis*
14 is also a secondary sexual characteristic utilised during the act of spawning.

15 Both Potter et al. (1983) and Glova (1995) attempted to rear migratory *G. australis* in the
16 laboratory through to spawning. In both studies, most lamprey matured 14-16 months after
17 entry into fresh water, but spawning was not documented. Although the male lamprey
18 developed the gular pouch, the female lamprey showed no pronounced external changes aside
19 from the obvious gravid abdomen. Male sea lamprey develop their rope at the final stage of
20 sexual maturation (Chung-Davidson et al. 2013). The absence of the rope tissue in mature
21 female *G. australis* in the studies of Potter et al. (1983) and Glova (1995) may therefore be
22 because the lamprey were not able to be reared through to the act of spawning. However, the
23 development time from migration to sexual maturity observed by Potter et al. (1983) and

1 Glova (1995) was consistent with that seen in the present study as in both 2013 and 2015
2 spawning took place 14 months after the lamprey migrated into the catchment.

3 Although the nesting habitat of *G. australis* shows considerable variation from Petromyzontid
4 lampreys, embryological development and early growth was similar to that seen in the sea
5 lamprey, which has a similar egg size at maturity (Piavis 1971; Potter et al.1983). Fecundity
6 was also on par with that previously measured in *G. australis* (mean fecundity 57,943; Potter
7 et al. 1983), and the expected fecundity of a large bodied species (Docker and Beamish
8 1991).

9 Early larval behaviour, however, differed from that seen in Petromyzontid lampreys as larval
10 *G. australis* remained in the nest for at least two weeks before dispersal, as opposed to
11 dispersing immediately post-hatching. The caudal region of *G. australis* larvae was adhesive,
12 providing a means of anchoring the larvae to the boulders within the nest. This may prevent
13 displacement downstream by currents or when disturbed by adult movements. It is not
14 currently known whether larvae excrete an adhesive substance or use another mechanism to
15 adhere to surfaces. In addition, the advantage to *G. australis* larvae by remaining in the nest
16 is also unknown and therefore early larval behaviour in this species warrants further
17 investigation.

18 In summary, this study documents the first nesting sites of a Southern Hemisphere lamprey
19 species. Presently, nest sites of *G. australis* have only been observed underneath large
20 boulders with the same habitat utilised across two years, but observations are still limited.
21 Data indicate that both male and female lamprey survive spawning and remain at the nest site
22 for over 105 days before mortality. These differences in reproductive behaviour compared
23 with Petromyzontidae lampreys may represent an evolutionary divergence between the two
24 families. A caveat of the present study is the low sample size and further investigations are

1 needed to determine if spawning habitat is consistent across *G. australis* populations, and to
2 elucidate what role male and female *G. australis* undertake during egg development.
3 However, knowledge of the reproductive ecology of *G. australis* is an important pre-cursor to
4 the conservation and management of this unique species. The results from this study improve
5 our understanding of this critical life-stage and will facilitate the protection and conservation
6 of *G. australis* in New Zealand.

7

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1 **References**

- 2 Bagenal, T.B., and Braum, E. 1978. Eggs and early life history. *In* Methods for assessment of
3 fish production in fresh waters. International Biological Programme Handbook No. 3. *Edited*
4 *by* T. B. Bagenal. Blackwell Scientific Publications Ltd, Oxford. pp 165-178.
- 5
- 6 Beamish, F.W.H. 1980. Biology of the North American anadromous sea lamprey,
7 *Petromyzon marinus*. Can. J. Fish. Aquat. Sci. **37**: 1924–1943.
- 8
- 9 Beumer, J. P. (1979). Feeding and movement of *Anguilla australis* and *A. reinhardtii* in
10 Macleods Morass, Victoria, Australia. J. Fish Biol. **14**: 573–592.
- 11
- 12 Bovee, K.D., and Milhous, R. 1978. Hydraulic simulation in instream flow studies: theory
13 and techniques. United States Fish and Wildlife Service, Cooperative Instream Flow Group,
14 Instream flow information paper 5. 131 p.
- 15
- 16 Chung-Davidson, Y-W, Priess, M.C., Yeh, C-Y., Brant, C.O., Johnson, N.S., Li, K.,
17 Nanlohy, K.G., Bryan, M.B., Brown, C.T., Choi, J., and Li, W. 2013. A thermogenic
18 secondary sexual character in male sea lamprey. J. Exp. Biol. **216**: 2702–2712.
- 19
- 20 Cochran, P.A., and Gripentrog, A.P. 1992. Aggregation and spawning by lampreys (genus
21 *Ichthyomyzon*) beneath cover. Env. Biol. Fish. **33** :381–387.
- 22
- 23 Cochran, P.A., and Lyons, J. 2004. Field and laboratory observations on the ecology and
24 behavior of the silver lamprey (*Ichthyomyzon unicuspis*) in Wisconsin. J. Fresh. Ecol. **19**:
25 245–253.

- 1
2 Docker, M.F., and Beamish W.H. 1991. Growth, fecundity, and egg size of least brook
3 lamprey, *Lampetra aepyptera*. Env. Biol. Fish. **31**: 219-227.
4
5 Duncan, M.J., and Woods, R.A. 2013. Water regulation. In Ecosystem services in New
6 Zealand – conditions and trends. Edited by J. R. Dymond. Manaaki Whenua Press, Lincoln,
7 New Zealand. pp 410-473.
8
9 Gardner, C., Coghlan, S.M., and Zydlewski, J. 2012. Distribution and abundance of
10 anadromous sea lamprey spawners in a fragmented stream: current status and potential range
11 expansion following barrier removal. North. Natural. **19**: 99–110.
12
13 Ghosh, S., Ajith Kumar, T.T., and Balasubramanian, T. 2012. Determining the level of
14 parental care relating fanning behavior of five species of clownfishes in captivity. Indian J.
15 Geo. Mar. Sci. **41**: 430-441.
16
17 Glova, G. 1995. The secret life of the lamprey. NIWA Water & Atmosphere **3**: 20-21.
18
19 Goodman, J., Dunn, N.R. Ravenscroft, P., Allibone, R., Boubée, J., David, B., Griffiths, M.,
20 Ling, N., Hitchmough, R., and Rolfe, J. 2014. Conservation status of New Zealand freshwater
21 fish, 2013. New Zealand Threat Classification Series 7.
22
23 Gunckel, S. L., Jones, K.K., and Jacobs, S.E. 2009. Spawning distribution and habitat use of
24 adult Pacific and western brook lampreys in Smith River, Oregon. In Biology, management,
25 and conservation of lampreys in North America. Edited by L. R. Brown, S. D. Chase, M. G.

- 1 Mesa, R. J. Beamish, and P. B. Moyle. American Fisheries Society Symposium 72, Bethesda,
2 Maryland, USA. pp 173-189.
3
- 4 Hardisty, M.W., and Potter, I.C. 1971. The general biology of adult lampreys. *In* The Biology
5 of Lampreys. *Edited by* M.W. Hardisty and I.C. Potter. Academic Press, London. pp 127–
6 206.
7
- 8 Heggenes, L. 1988. Effects of short-term flow fluctuations on displacement of, and habitat
9 use by, brown trout in a small stream. *Trans. Am. Fish. Soc.* **117**: 336-344.
10
- 11 Janvier, P. 2007. Living primitive fishes and fishes from deep time. *In* Primitive fishes.
12 Volume 26. Fish physiology. *Edited by* D. J. McKenzie, A. P. Farrell, and C. J. Brauner.
13 Academic Press, San Diego, USA. pp 1 – 51.
14
- 15 Jellyman, D.J., Glova, G.J. and Sykes, J.R.E. 2002. Movements and habitats of adult lamprey
16 (*Geotria australis*) in two New Zealand waterways. *N.Z. J. Mar. Fresh. Res.* **36**: 53-65.
17
- 18 Johnson, N.S., Buchinger, T.J., and Li, W. 2015. Reproductive Ecology of Lampreys. *In*
19 Lampreys: Biology, Conservation and Control. Fish & Fisheries Series, 37. *Edited by* M. F.
20 Docker. Springer, Netherlands. pp 265-303.
21
- 22 Johnston, P.G., Potter, I.C., and Robinson, E.S. 1987. Electrophoretic analysis of populations
23 of the southern hemisphere lampreys *Geotria australis* and *Mordacia mordax*. *Genetica* **74**:
24 113-117.
25

- 1 Jowett, I.G., and Richardson, J. 1989. Effects of a severe flood on instream habitat and trout
2 populations in seven New Zealand rivers. N.Z. J. Mar. Fresh. Res. **23**: 11-17.
3
- 4 Jowett, I.G., and Richardson, J. 1990. Microhabitat preferences of benthic invertebrates in a
5 New Zealand river and the development of in-stream flow habitat models for *Deleatidium*
6 spp. N.Z. J. Mar. Fresh. Res. **24**: 19–30.
7
- 8 Jowett, I.G., Richardson, J. Biggs, B.J.F., Hickey, C.W., and Quinn, J.M. 1991. Microhabitat
9 preferences of benthic invertebrates and the development of generalised *Deleatidium* spp.
10 Habitat suitability curves, applied to four New Zealand rivers. N.Z. J. Mar. Fresh. Res. **25**:
11 187–199.
12
- 13 Kelso, J.R.M., and Glova, G.J. 1993. Distribution, upstream migration and habitat selection
14 of maturing lampreys, *Geotria australis*, in Pigeon Bay Stream, New Zealand. Aust. J. Mar.
15 Fresh. Res. **44**: 749-759.
16
- 17 Kelso, J.R.M., and Todd, P.R. 1993. Instream size segregation and density of *Geotria*
18 *australis* ammocoetes in two New Zealand streams. Ecol. Fresh. Fish **2**: 108-115.
19
- 20 Korolyev, V.V., and Reshetnikov, Yu.S. 2008. Rare species of Cyclostomata and fish in the
21 upper Oka Basin and within Kaluga Oblast. J. Ichthyol. **48**: 583-595.
22
- 23 Maskell, F.G. 1929. On the New Zealand lamprey, *Geotria australis* Gray. Part 1. Biology
24 and life history. Trans. Proc. N.Z. Inst. **60**: 167-207.
25

- 1 McDowall, R.M. 1990. New Zealand freshwater fishes: a natural history and guide.
2 Heinemann-Reed, Auckland, New Zealand.
3
- 4 McDowall, R.M. 2011. Ikawai. Freshwater fishes in Māori culture and economy, Canterbury
5 University Press, Christchurch, New Zealand.
6
- 7 McDowall, R.M., and Eldon, G.A. 1997. Reproductive cycling and fecundity estimation in
8 the upland bully. *J. Fish Biol.* **51**: 164-179.
9
- 10 McEwan, A.J., and Joy, M.K. 2013. Responses of three PIT-tagged native fish species to
11 floods in a small, upland stream in New Zealand, N.Z. *J. Mar. Fresh. Res.* **47**: 225-234.
12
- 13 Monette, S.N., and Renaud, C.B. 2005. The gular pouch in northern hemisphere parasitic
14 lampreys (Petromyzontidae). *Can J Fish Aquat Sci* **83**: 527–535.
15
- 16 Piavis, G.W. 1971. Embryology. *In The Biology of Lampreys. Edited by M. W. Hardisty, and*
17 *I. C. Potter. Academic Press, London, UK. pp 361-400.*
18
- 19 Pletcher, F.T. 1963. The life history and distribution of lampreys in the Salmon and certain
20 other rivers in British Columbia, Canada. Ph.D. thesis, University of British Columbia,
21 Vancouver.
22
- 23 Poff, N.L., and Ward, J.V. 1989. Implications of streamflow variability and predictability for
24 lotic community structure: a regional analysis of streamflow patterns. *Can. J. Fish. Aquat.*
25 *Sci.* **46**: 1805–1817.

- 1
- 2 Poff, N.L., Olden, J.D., Pepin, D.M., and Bledsloe, B.P. 2006. Placing global stream flow
3 variability in geographic and geomorphic contexts. *River Res. Appl.* **22**: 149–166.
- 4
- 5 Potter, I.C. 1980. Ecology of larval and metamorphosing lampreys. *Can. J. Fish. Aquat. Sci.*
6 **37**: 1641–1657.
- 7
- 8 Potter, I.C., and Welsch, U. 1997. The structure of the gular pouch of mature males of the
9 lamprey *Geotria australis*. *Acta Zool.* **78**: 97–106.
- 10
- 11 Potter, I.C., Hilliard, R.W., Bird, D.J., and Macey, D.J. 1983. Quantitative data on the
12 morphology and organ weights during the protracted spawning-run period of the Southern
13 Hemisphere lamprey *Geotria australis*. *J. Zool.* **200**: 1-20.
- 14
- 15 Potter, I.C., Gill, H.S., Renaud, B.B. and Haoucher, D. 2015. The Taxonomy, Phylogeny, and
16 Distribution of Lampreys. *In* *Lampreys: Biology, Conservation and Control*. Fish & Fisheries
17 Series, 37. Edited by M. F. Docker. Springer, Netherlands. pp 35-73.
- 18
- 19 Reyes, R.C. 2008. Embryogenesis and Ammocoete Morphological Development of the
20 Pacific Lamprey (*Entosphenus tridentatus* Gairdner, 1836) from the American River,
21 California. Tracy Technical Bulletin 2008-3. U.S. Department of the Interior – Bureau of
22 Reclamation Technical Service Center.
- 23

1 Silva, S., Gooderham, A., Forty, M., Morland, B., and Lucas, M.C. 2014. Egg drift and
2 hatching success in European river lamprey *Lampetra fluviatilis*: is egg deposition in gravel
3 vital to spawning success? Aquat. Conserv. DOI: 10.1002/aqc.2486.
4
5 Stone, J. 2006. Observations on nest characteristics, spawning habitat, and spawning behavior
6 of Pacific and western brook lamprey in a Washington stream. North. Nat. **87**: 225–232.
7

Draft

1 Table 1. Physical habitat features of the nest sites ($n = 6$) including the size of the boulder
 2 eggs were found beneath. The habitat features associated with maturing lamprey ($n = 18$) are
 3 also presented, including the boulder size utilised as cover. The substrate index is based on
 4 the modified Wentworth particle size scale and ranges from vegetation (1) to bedrock (8).
 5 Significant differences between means are signified by asterisks (Mann-Whitney U tests, $P <$
 6 0.05).

Habitat features	Nesting fish		Maturing fish	
	Mean ($\pm$ SE)	Max	Mean ($\pm$ SE)	Max
Boulder size (l) (m)*	0.70 (0.07)	0.83	0.49 (0.03)	0.75
Boulder size (w) (m)*	0.44 (0.05)	0.55	0.32 (0.03)	0.53
Water depth (m)	0.10 (0.01)	0.20	0.13 (0.02)	0.32
Water velocity (m s^{-1})	0.20 (0.04)	0.44	0.18 (0.02)	0.45
Substrate index	6.50 (0.06)	6.7	6.17 (0.09)	6.95
Wetted width (m)	2.14 (0.14)	2.75	2.38 (0.12)	4.00

7

8

- 1 Fig. 1. Study site location. a) the South Island of New Zealand showing the location of Banks
2 Peninsula. b) Banks Peninsula with the location of the Okuti River and Kinloch Stream
3 highlighted. c) the Okuti River and Kinloch Stream study sites on the eastern side of Lake
4 Wairewa. Fish release sites are marked by circles with lamprey captured within
5 approximately 200 m downstream of each release site. d) Kinloch Stream showing the
6 location of the nest sites documented in 2013 (white stars) and 2015 (black stars).
- 7 Fig. 2. *G. australis* nest site. a) Boulder covering the nest is indicated by the arrow, b)
8 Overturned boulder exposing egg mass. Inset shows a close-up of the egg mass.
- 9 Fig. 3. Sequence of still frames showing a male *G. australis* wrapping himself around the egg
10 mass after intrusion of the camera on 18 November 2015.
- 11 Fig. 4. Post-spawning female *G. australis* with a rope evident along the dorsal ridge. Arrows
12 indicate lateral edges of the rope.
- 13 Fig. 5. Newly hatched *G. australis* (a) clustered between boulders 10 days post-hatching on
14 18 December 2015 and (b) still attached to boulders two weeks post-hatching on 23
15 December 2015 adjacent to the male lamprey, whose folded gular pouch is evident.
- 16 Fig. 6. Cluster of *G. australis* 10 days post-hatching showing the caudal region of larvae
17 adhering to debris.

Fig. 1. Study site location. a) the South Island of New Zealand showing the location of Banks Peninsula. b) Banks Peninsula with the location of the Okuti River and Kinloch Stream highlighted. c) the Okuti River and Kinloch Stream study sites on the eastern side of Lake Wairewa. Fish release sites are marked by black circles with lamprey captured within approximately 200 m downstream of each release site. d) Kinloch Stream showing the location of the nest sites documented in 2013 (white stars) and 2015 (black stars).

296x419mm (300 x 300 DPI)

Fig. 2. *G. australis* spawning nest. a) Boulder covering the nest is indicated by the arrow, b) Overturned boulder exposing egg mass. Inset shows a close-up of the egg mass.

87x134mm (300 x 300 DPI)

Fig. 3. Sequence of still frames showing a male *G. australis* wrapping himself around the egg mass after intrusion of the camera on 18 November 2015.

97x52mm (300 x 300 DPI)

Fig. 4. Post-spawning female *G. australis* with a rope evident along the dorsal ridge. Arrows indicate lateral edges of the rope.

200x156mm (300 x 300 DPI)

Fig. 5. Newly hatched *G. australis* (a) clustered between boulders 10 days post-hatching on 18 December 2015 and (b) still attached to boulders two weeks post-hatching on 23 December 2015 adjacent to the male lamprey, whose folded gular pouch is evident.

182x73mm (300 x 300 DPI)

Draft

Fig. 6. Cluster of *G. australis* 10 days post-hatching showing the caudal region of larvae adhering to debris.

1828x1219mm (72 x 72 DPI)