First records of egg masses of *Nototodarus gouldi* McCoy, 1888 (Mollusca: Cephalopoda: Ommastrephidae), with comments on egg-mass susceptibility to damage by fisheries trawl

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Abstract The egg mass and embryos of the ommastrephid squid *Nototodarus gouldi* McCoy, 1888 are reported for the first time, their identity confirmed by mitochondrial 16S rDNA sequence determination. The egg mass is a free-floating gelatinous sphere of at least 1.5 m diameter and contains an estimated several thousand randomly distributed eggs; similar egg masses recorded from north-eastern New Zealand waters of 1.0–2.0 m diameter are reported. Observed fluctuations in populations for this and other squid species may be a partial result of trawl damage to the egg masses.

Keywords *Nototodarus*; Cephalopoda; Ommastrephidae; egg mass; trawling

INTRODUCTION

In recent years, nine reports of large gelatinous spheres sighted off north-eastern New Zealand have been brought to our attention. Between November 2001 and March 2003, eight of these spheres ranging from 1.0 to 2.0 m diameter were reported by divers off the Poor Knights Islands (Fig. 1); a further sighting was reported from Deep Water Cove, Bay of Islands, in November of 1997 (Table 1).

In photographs, these structures bore striking resemblance to egg masses from laboratory-spawned ommastrephid squid genera *Todarodes* and *Illex* (O’Dor & Balch 1985, Bower & Sakurai 1996). Accordingly, the gelatinous spheres were tentatively identified as the egg masses of an unknown species of squid.

This paper reports the most recently observed sphere, a 1.5 m diameter mass encountered in the entrance to Rikoriko cave, Poor Knights Islands on 26 March 2003, for which excellent photographs, film, and tissue samples are available. The egg mass sample contained embryonic squid, but the embryos were too undeveloped to attribute to any recognised taxon based on morphology (mantle and funnel-locking cartilages, tentacles and arm suckers were still undeveloped). The egg mass disintegrated 2 days later, resulting in the loss of all embryos. As a precautionary measure, in the event the embryos could not be hatched, five had earlier been preserved in 90% ethanol.

Egg masses have been described or their type is known for the following squid in New Zealand waters: two *Sepioteuthis* and three *Sepioloidea* species, both of which deposit and attach eggs in gelatinous clusters to the seabed; seven locally distributed enoploteuthid squid (Enoploteuthidae) and one *Brachioteuthis* (Brachioteuthidae) species, all of which release eggs individually into the plankton (Young & Harman 1985; Young et al. 1985); and a commercially important squid, *Thysanoteuthis rhombus* (Thysanoteuthidae), for which the egg mass is described as a cylindrical structure of 0.6–0.7 m length and 0.15–0.2 m diameter (Sanzo 1929). The egg masses of the other 76 (approximate) species of squid represented in museum collections from New Zealand waters were unknown.

In the present study we have used mitochondrial DNA (mtDNA) sequences to identify the first-known egg mass of the commercially important
ommatrephid squid genus *Nototodarus*, and describe the first structure of its type from New Zealand waters. Furthermore, in light of the tenuous nature of the egg mass, its size and bathymetric distribution, we take the opportunity to express concern regarding the inadvertent damage to or destruction of these spheres by trawlers passing through spawning aggregations of squid.

**METHODS**

In 1981, the ocean surrounding the Poor Knights Islands, off the East Coast of Northland, was established as New Zealand’s second marine reserve. This reserve extends for 800 m offshore around these islands; the taking or disturbing of any marine life within the reserve is prohibited. In anticipation of summer egg-mass sightings in this region, a special permit issued under the Marine Reserves Act by the New Zealand Department of Conservation, after consultation with the Ngati Wai Trust Board, was granted. This allowed divers to collect a sample of ~50 eggs from an egg mass from within the reserve, to enable species identification (through both morphological and DNA analyses). On 26 March 2003 an egg mass of 1.5 m diameter was sighted, and a sample of ~50 eggs was removed from it. Within 10 h of collection the egg and gel matrix was transported ashore in a container of water, returned to Auckland (AUT) and placed within a fine-meshed bag suspended within a 200-litre hexagonal tank, in which the seawater was maintained at a constant temperature of 20°C. The status of the gel and

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**Table 1** North-eastern New Zealand records of gelatinous squid egg spheres.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Depth</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 Mar 2003</td>
<td>Rikoriko Cave</td>
<td>22 m; 20°C</td>
<td>1.5 m(^1)</td>
</tr>
<tr>
<td>17 Mar 2003</td>
<td>Middle Arch</td>
<td>&lt;30 m</td>
<td>Not cited(^2)</td>
</tr>
<tr>
<td>16 Dec 2002</td>
<td>Trevor’s Rocks</td>
<td>&lt;30 m</td>
<td>~1 m(^3)</td>
</tr>
<tr>
<td>9 Apr 2002</td>
<td>Anne’s Reef</td>
<td>14 m</td>
<td>~1 m(^2)</td>
</tr>
<tr>
<td>23 Mar 2002</td>
<td>Butterfish Bay</td>
<td>30 m</td>
<td>~2 m(^4)</td>
</tr>
<tr>
<td>23 Mar 2002</td>
<td>Landing Bay Pinnacle</td>
<td>Not cited</td>
<td>Not cited(^4)</td>
</tr>
<tr>
<td>5 Feb 2002</td>
<td>Eastern side of Ngaio Rock</td>
<td>10 m</td>
<td>1.5–2.0 m(^5)</td>
</tr>
<tr>
<td>Nov 2001</td>
<td>Middle Arch</td>
<td>15 m</td>
<td>~1.3 m(^6)</td>
</tr>
<tr>
<td>Dec 1997</td>
<td>Deep Water Cove, Bay of Islands</td>
<td>12 m</td>
<td>1.0 m(^7)</td>
</tr>
</tbody>
</table>

Observations courtesy of: Pete Mesley\(^1\); Wade Doak\(^2\); Haydon Spencer\(^3\); Ross & Diane Armstrong\(^4\); Tony & Jenny Enderby\(^5\); Dave Abbot\(^6\); Toby Bernard\(^7\).
Fig. 2  Egg mass, 2 m diameter, 30 m depth, Butterfish Bay, Poor Knights Islands (photograph courtesy Ross Armstrong).

Fig. 3  Close up, egg mass, 2 m diameter, 30 m depth, Butterfish Bay, Poor Knights Islands (photograph courtesy Ross Armstrong).

Fig. 4  Egg mass, 1.5–2.0 m diameter, 10 m depth, eastern side of Ngaio Rocks, Poor Knights Islands (photograph courtesy Tony Enderby).

Fig. 5  Egg mass, 1.5 m diameter, 22 m depth, entrance to Rikoriko Cave, Poor Knights Islands (photograph courtesy Pete Mesley).

Fig. 6  Embryos removed from 1.5 m diameter egg mass, 22 m depth, entrance to Rikoriko Cave, Poor Knights Islands (photograph K. Bolstad).
embryonic tissues was monitored daily. Five embryos were removed from the gel matrix and preserved in 90% ethanol solution for DNA analysis.

As GenBank mitochondrial 16S rDNA sequences were not available for *N. gouldi*, voucher tissues were sourced for comparative purposes. Only male *N. gouldi*, identified on grounds of hectocotylus detail (sensu Smith et al. 1987), were used for genetic comparison, as female *N. gouldi* and *N. sloanii* cannot be reliably differentiated (they are sympatric in the area from which fresh *Nototodarus* tissues were sourced, the Wairarapa Coast, south-eastern North Island, New Zealand; Smith et al. 1987).

DNA was subsequently purified from four of the embryos and one adult male *N. gouldi* using standard procedures of phenol-chloroform extraction and ethanol precipitation (Sambrook et al. 1989). Mock extractions were performed at the same time to control for cross-contamination of samples. DNA yields from each sample were quantified using a Hoefer DyNA Quant 200 fluorometer. The mitochondrial 16S rDNA gene was amplified with the primers L-16SAr (5'-CGCGTCTGATCTACGAT-3') and H-16SBr (5'-CCGGTCTGAACTCTGATCAT-3') (Piertney et al. 2003). For polymerase chain reaction (PCR) amplifications we used 25 µl volumes using 200 ng of DNA and 10 mM Tris pH 8.0, 50 mM KCl, 1.5 mM MgCl2, 0.4 µM of each primer, 200 µM of each dNTP and one unit of Taq DNA Polymerase (Roche). Thermal cycling was carried out on a Hybaid Omn-E at 94°C 10 s, 60°C 10 s, and 7°C 45 s for 30 cycles. The resulting PCR products were purified using the High Pure PCR Product Purification kit (Roche), sequenced using the PRISM® BigDye™ Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems), and analysed on a 3730 automated sequencer (Applied Biosystems). DNA sequences were deposited in GenBank under the accession number AY380810.

The four sequences from the egg mass were aligned to known sequences retrieved from the database with GenBank. The number of pairwise differences between all sequences was estimated using PAUP*4.0b10 (Swofford 2002).

RESULTS

DNA was successfully extracted and consistently amplified from four embryos. The eggs measured approximately 2 mm in diameter and yielded 5.8–6.3 µg of total genomic DNA. The replicate egg samples all produced identical sequences. The resulting DNA sequences from the four embryos and the known *N. gouldi* sample were aligned to 60 squid sequences that were retrieved from the database GenBank. Using the genetic distance analysis the egg samples were unambiguously assigned to the species *N. gouldi*. The number of pairwise sequence differences between the egg samples and the five most closely related species were *N. gouldi* = 0, *N. hawaiensis* = 16, *Todarodes filippovae* = 18, *Illex argentinus* = 19, *Sepioteuthis australis* = 25, and *Sthenoteuthis oualaniensis* = 31.

DISCUSSION

Two species of *Nototodarus* live in New Zealand waters: *N. gouldi* and *N. sloanii*. Although tissues of *N. sloanii* could not be procured in time for inclusion in this analysis, because it is the southernmost of the two species found in New Zealand waters it is not possible that *N. sloanii* released the large egg spheres herein reported from north-eastern New Zealand. It is excluded from comparison on biogeographic grounds.

Mattlin et al. (1985) suggest that *Nototodarus* spawns in the upper 100 m of the water column (Uozumi et al. 1995), a theory herein supported, in part, by the identification of *N. gouldi* egg masses in the upper 10–30 m.

McGrath & Jackson (2002) estimated mature female *N. gouldi* oviduct-egg counts in the range 2176–82 395 and concluded that this squid releases eggs in series of small clutches, rather than in one terminal spawning event. Although the density of eggs in the egg mass here reported could not be accurately determined, we estimated that it contained at least several thousand eggs. This low estimate lends support to McGrath & Jackson’s suggestion that *N. gouldi* does in fact spawn more than once. However, for *N. gouldi* to spawn several times it must also mate several times. Balch et al. (1985) reported relative fertilisation rates for eggs spawned by a captive *Illex illecebrosus* (Ommastrephidae), as 15% for the first of two masses, to <1% for the second. The reduced success for the second mass was attributed to sperm depletion, because most spermatophores were discharged to fertilise the first mass. Although Bower & Sakurai (1996) report laboratory-spawned egg masses of *Todarodes pacificus* with fertilisation rates of 90–95%, these females died immediately after spawning, with 93 000–110 000 eggs remaining in their oviducts.
Given the known range of intraspecific variation in egg mass diameter described for ommastrephid squid (*Illex illecebrosus* 0.4–1.0 m (Balch et al. 1985), *Todarodes pacificus* 0.4–0.8 m (Bower & Sakurai 1996)), it is possible that the nine spheres reported from north-eastern New Zealand waters all belonged to *N. gouldi*. If so the species must spawn off north-eastern New Zealand from late November to early April. Since the egg masses of the 75 other species of squid (excluding *N. gouldi* and the aforementioned taxa) are unknown, it is not clear whether or not these egg masses are truly conspecific.

Uozumi & Förch (1995) determined that most larval *N. gouldi* in central and southern New Zealand waters were hatched during the months of April and June. Artificially fertilised *Illex argentinus* eggs hatched in 5–16 days (Sakai et al. 1998), and those of *I. illecebrosus* hatched 6–16 days post-spawning (Durward et al. 1980; O’Dor et al. 1982). Temperature determined the rate of embryonic development in both species. Eggs of the congeneric *Todarodes pacificus* hatched in 4–6 days (Bower & Sakurai 1996), and 7 days (Watanabe et al. 1996). If embryos of *N. gouldi* develop at comparable rates, then the egg-mass life span is short and paralarval squid should start appearing in the water column in December, extending through until mid April. These estimated hatching dates are considerably earlier than those proposed for this species from central and southern New Zealand waters (Uozumi & Förch 1995), indicating that *N. gouldi* has a separate summer-spawning population in waters off and north of the Poor Knights Islands.

**CONSERVATION**

Development of eggs isolated from the egg mass gel in *Illex illecebrosus* has been described as abnormal, compared with those developing within intact egg masses (Balch et al. 1985). Collision with a trawl, or retention of the egg mass in the net itself, may destroy the structural integrity of the mass and viability of developing embryos and/or expose thousands of fragmented embryos to predation by fish and planktonic organisms. After the outer surface of the *N. gouldi* egg mass reported here was cut for sample collection, video footage revealed near-instantaneous predation on the inner gel and egg matrix by reef fish. Moreover, crustaceans, protozoans, and bacteria rapidly infested the egg mass once its outer layer was damaged (Bower & Sakurai 1996).

Since *Nototodarus* species are locally caught by both jig and trawl (Uozumi & Förch 1995), it is likely that any trawl fishery targeting spawning aggregations will inadvertently damage or destroy any egg masses present, killing the developing embryos. This impact on the earliest stages of the life cycle could be a contributing factor to fluctuating squid populations, both in New Zealand waters and on an international scale. Serious consideration should be given to introducing regulations that would limit fishing activity amid spawning squid aggregations solely to jigging techniques.

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**REFERENCES**


