

## The use of egg shells to infer the historical presence of copepods in alpine lakes

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### Abstract

Copepods (Class Crustacea, Order Copepoda) are rarely included in paleoecological studies of lakes because they lack long-lasting exoskeletal remains. We describe the remains of eggs (egg shells) from *Hesperodiaptomus* copepods that are well preserved and abundant in alpine lake sediments. We demonstrate that the egg shells are the remains of *Hesperodiaptomus* eggs based on (i) the similar size and morphology of egg shells collected from sediments and those produced from the hatching of eggs obtained from laboratory-maintained *Hesperodiaptomus*, and (ii) the finding that diapausing eggs collected from lake sediments and hatched in the laboratory produced copepod nauplii that were morphologically indistinguishable from those hatched from eggs produced by laboratory-maintained *Hesperodiaptomus*. Egg shells were approximately two orders of magnitude more abundant in sediment cores than were viable diapausing eggs, making egg shells superior to viable diapausing eggs for quantifying the historical presence and abundance of *Hesperodiaptomus*. These results have important implications for alpine lake restoration as egg shells can be used to identify lakes in which *Hesperodiaptomus* was eliminated by fish introductions but has failed to return after fish eradication, lakes in which the pre-disturbance conditions are likely to be restored only by reintroductions of this important taxon.

### Introduction

Remains from an array of invertebrate taxa are often well preserved in lake sediments, and are commonly used to reconstruct historical zooplankton and benthic invertebrate community composition. Community reconstructions typically do not include copepods (Class Crustacea, Order Copepoda), however, because of the lack of long-lasting exoskeletal remains (Frey, 1964). Fossil egg sacs from one species of *Diaptomus* have been described (Bennike, 1998), but may be of limited utility in historical reconstructions because of their low

density in lake sediments ( $< 0.004$  sacs  $\cdot$  cm<sup>-3</sup>; Bennike, 1998). Copepod diapausing eggs (Hairston et al., 1995) have some utility in reconstructing historical copepod presence (Parker et al., 1996), but diapausing eggs are depleted from the sediments over time through hatching, predation, and mortality (Hairston et al., 1995; Parker et al., 1996). As a result, diapausing eggs of a particular species may be rare or absent from the sediments of lakes that harbored the species in the past (Parker et al., 1996). Even when diapausing eggs are abundant enough to provide an historical record of species presence, the depletion of diapausing eggs over time

makes their use as an index of past abundance problematic. Parker et al. (1996) used empty egg cases to estimate the past abundance of *Hesperodiaptomus arcticus* Light (synonymous with *Diaptomus arcticus* Marsh) diapausing eggs, but provided no quantitative evidence that egg cases were in fact from *H. arcticus* eggs.

In the Sierra Nevada (California, USA), the stocking of fish into historically fishless alpine lakes has resulted in the elimination of several species of large zooplankton, including *Daphnia middendorffiana* Fischer and *Hesperodiaptomus shoshone* Light (synonymous with *Diaptomus shoshone* Forbes) (Stoddard, 1987; Knapp, 1996; Bradford et al., 1998). Similar effects of fish introductions on alpine lake zooplankton communities have also been reported for the Rocky Mountains (Anderson, 1980; Carlisle & Hawkins, 1998; McNaught et al., 1999). As part of a study of the recovery of *Hesperodiaptomus* in Sierra Nevada lakes after fish removal, we sought to establish whether these copepods were present historically in a set of lakes that were stocked with fish in the past but are now fishless, yet do not currently contain *Hesperodiaptomus*. Such reconstruction is hampered by a paucity of data on zooplankton composition prior to fish introductions. During our analyses of sediment cores from these lakes, we found what appeared to be the shells of hatched (or otherwise destroyed) *Hesperodiaptomus* diapausing eggs. If true, these egg shells could be used to ascertain the historical presence of *Hesperodiaptomus*, and allow us to distinguish between lakes in which their current absence is due to a failure to recolonize and lakes in which they were never present.

Although some zooplankton species produce both diapausing (delayed hatching) and subitaneous (rapidly hatching) eggs (Hairston & Olds, 1984), it is unknown whether *Hesperodiaptomus* produce both types of eggs. We refer to *Hesperodiaptomus* eggs as 'diapausing' eggs because we found them buried > 2 cm deep in lake sediments (see Results) and these eggs were therefore at least several years old. In addition, *Hesperodiaptomus* eggs incubated in the laboratory (see Results) often required several months to hatch.

The primary objective of this paper is to provide evidence that the egg shells found in sediments of Sierra Nevada alpine lakes are in fact the remains of *Hesperodiaptomus* eggs. A secondary objective is to compare the efficacy of egg shells versus viable diapausing eggs in allowing the reconstruction of historical presence and abundance of this taxon.

## Methods

We obtained one sediment core from each of eight lakes located in the John Muir Wilderness, Inyo National Forest, California, USA. Seven of the lakes are in Humphreys Basin (37° 27'N, 118° 42'W) and one is in the Rock Creek drainage (37° 15'N, 118° 45'W). Four lakes are fishless and currently harbor populations of *Hesperodiaptomus shoshone* and four contain introduced golden trout (*Oncorhynchus mykiss aguabonita*) and lack *Hesperodiaptomus* (Knapp, unpublished data). Sediment cores were collected through the ice in June 1998 with a piston corer (Fisher et al., 1992), or in August–September 1998 by a diver using a 7 cm diameter core barrel identical to that used in the piston corer. Cores were taken from the deepest point in each lake. The first 10 cm of each core were sectioned at 1 cm increments in the field. Two cores were less than 10 cm long, and in these cases the entire core was sectioned. For two of the six cores that were longer than 10 cm, we also collected a 2 cm thick section from the 20–22 cm core depth. The outermost 5 mm of each section was trimmed off and discarded to eliminate contamination between sections caused by material dragging along the wall of the core barrel. The individually-bagged core sections were refrigerated at 4 °C until they were processed.

The contents of all core sections were searched for egg shells and viable diapausing eggs within 1–30 days of core collection. Prior to enumeration of egg shells and viable diapausing eggs, the sediment making up each section was suspended in water, deflocculated using a sonicator, and filtered through a 150 µm sieve (diameters of eggs and egg shells ranged from 155–220 µm; eggs:  $n = 146$ ; Knapp & Sarnelle, unpublished data; egg shells: see Results). The contents of the sieve were searched for intact diapausing eggs and egg shells under a stereo-dissecting microscope at 10–40x. Intact diapausing eggs were characterized by the red, orange, or brown pigmentation of the embryo and all were considered to be viable (Hairston et al., 1995; Parker et al., 1996; Knapp, pers. observ.). The entire sample was processed unless egg shell densities were very high. In the latter situation, the sample was split 1–3 times using a Folsom plankton splitter. To ensure that the 150 µm sieve was fine enough to retain all intact eggs and egg shells, we filtered two core sections that contained abundant egg shells through the 150 µm sieve and then through a 75 µm sieve. We then searched the contents of the 75 µm sieve for eggs or egg shells. No eggs or egg shells were found.

To determine whether egg shells were the remains of *Hesperodiaptomus* diapausing eggs, we compared the structure and size of egg shells obtained from cores with egg shells resulting from the hatching of diapausing eggs produced by laboratory-reared *Hesperodiaptomus* females. Gravid *Hesperodiaptomus shoshone* females were collected from one of the study lakes and maintained in the laboratory until they dropped their egg sacs. Egg sacs were then placed in filtered lake water and held in an incubator at 15 °C. Eggs ( $n = 105$ ) were checked for hatching approximately every two weeks. Egg shells from lake sediments and egg shells resulting from the hatching of eggs obtained from laboratory-reared *Hesperodiaptomus* females were measured for maximum diameter using a computer-based image analysis system. Although eggs still enclosed within the egg case are often oblong (Figure 1A), eggs and egg shells from lake sediments and from laboratory-reared females were found singly and without a surrounding egg case. These eggs and egg shells were highly symmetrical.

We also made qualitative comparisons between nauplii that hatched from intact diapausing eggs collected from lake sediment cores ( $n = 55$ ) and nauplii that hatched from eggs produced by laboratory-reared *Hesperodiaptomus* females (Pennak, 1989: 415, Figure 6). Diapausing eggs collected from lake sediments were maintained using the same procedures as described for eggs produced by laboratory-reared females. Given the difficulty of identifying diaptomid nauplii to species, the primary purpose of these comparisons was to provide evidence that the eggs collected from sediment cores were in fact copepod eggs (based on the production of copepod nauplii) and thereby eliminate the remote

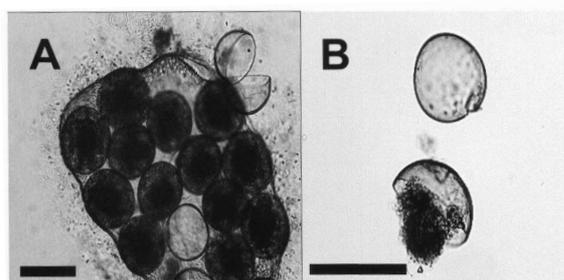


Figure 1. Photographs of (A) an egg sac from a laboratory-reared *Hesperodiaptomus* female containing both intact diapausing eggs (dark spheres) and egg shells (transparent hemispheres) resulting from hatched eggs, and (B) typical egg shells found in lake sediments. The scale bar in the lower left corner of each photograph is 200  $\mu\text{m}$  long.

possibility that these eggs were produced by a non-copepod lake-dwelling invertebrate.

## Results

The hatching of eggs obtained from laboratory-reared *Hesperodiaptomus* females resulted in two indistinguishable hemispherical shells (Figure 1A). Visually, these egg shells were identical to shells found in cores (Figure 1B). Shells from cores and from the hatching of eggs obtained from laboratory-reared *Hesperodiaptomus* females had mean diameters of 188 and 185  $\mu\text{m}$  respectively (Figure 2) and were not significantly different (two-tailed  $t$  test:  $n_{\text{core}} = 47$ ,  $n_{\text{lab}} = 41$ ,  $df = 86$ ,  $P > 0.2$ ). The size distributions of shells from cores and laboratory-hatched eggs were also not significantly different (Kolmogorov-Smirnov test:  $ks = 0.204$ ,  $P > 0.25$ ). In addition, 20 of the diapausing eggs collected

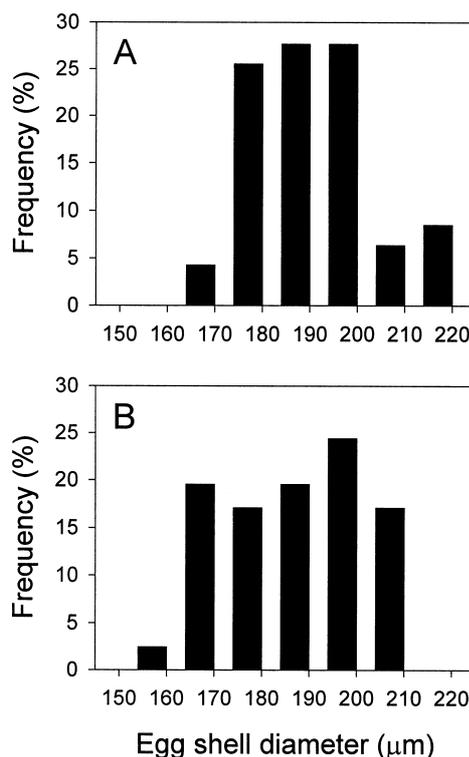


Figure 2. Histograms showing the size distribution of egg shells (A) from sediment cores ( $n = 47$ ), and (B) from eggs obtained from laboratory-reared *Hesperodiaptomus* and hatched ( $n = 41$ ).

from lake sediments hatched, and all produced copepod nauplii. These nauplii were qualitatively indistinguishable from the nauplii produced by the 21 diapausing eggs collected from laboratory-reared females that hatched.

Egg shells were found in cores from all of the eight study lakes (Figure 3), and densities in sections from the 0–10 cm core depth ( $n = 75$ ) ranged from 0–149 shells  $\cdot \text{cm}^{-3}$  (median = 4 shells  $\cdot \text{cm}^{-3}$ ). Viable diapausing eggs were found in cores from all four fishless lakes that currently contain *Hesperodiaptomus* (Figure 3A–D), but in only two of the four cores from fish-containing lakes that currently lack *Hesperodiaptomus* (Figures 3E,F). In addition, viable diapausing eggs were always sparser than egg shells ( $n = 75$ , range = 0–1.4 eggs  $\cdot \text{cm}^{-3}$ , median = 0 eggs  $\cdot \text{cm}^{-3}$ ). No viable eggs were found in the two 20–22 cm core slices; egg shells were present at a density of 3 shells  $\cdot \text{cm}^{-3}$  (for cores shown in Figures 3E, F).

## Discussion

Egg shells found in cores were of similar appearance and size to those produced from diapausing eggs collected from *Hesperodiaptomus* females maintained in the laboratory, all laboratory-maintained diapausing eggs that hatched produced copepod nauplii, and

nauplii from eggs collected from lake sediments and from eggs collected from laboratory-reared *Hesperodiaptomus* females were similar in appearance and size. Together, these observations provide strong evidence that the egg shells found in the sediments of our study lakes are the remains of *Hesperodiaptomus* diapausing eggs. No other copepods found in alpine Sierra Nevada lakes are large enough to have produced eggs of this size (Stoddard, 1987; Knapp, unpubl. data).

Egg shells were approximately two orders of magnitude more abundant in sediment cores than were viable diapausing eggs. As a result, egg shells are likely to be more useful than viable diapausing eggs for quantifying the historical presence and abundance of *Hesperodiaptomus*. Indeed, two of the four fish-containing study lakes which currently lack *Hesperodiaptomus* would have been misclassified as lacking *Hesperodiaptomus* historically if this determination was made solely on the presence of viable diapausing eggs.

The presence of *Hesperodiaptomus* egg shells in the 20–22 cm core sections suggests that egg shells are long-lived in alpine lake sediments. In one of the two cores for which we analyzed a core section from the 20–22 cm depth, we encountered discrete layers of volcanic ash (tephra) at 17 and 41 cm below the sediment surface. These tephra layers may correspond to the two tephra

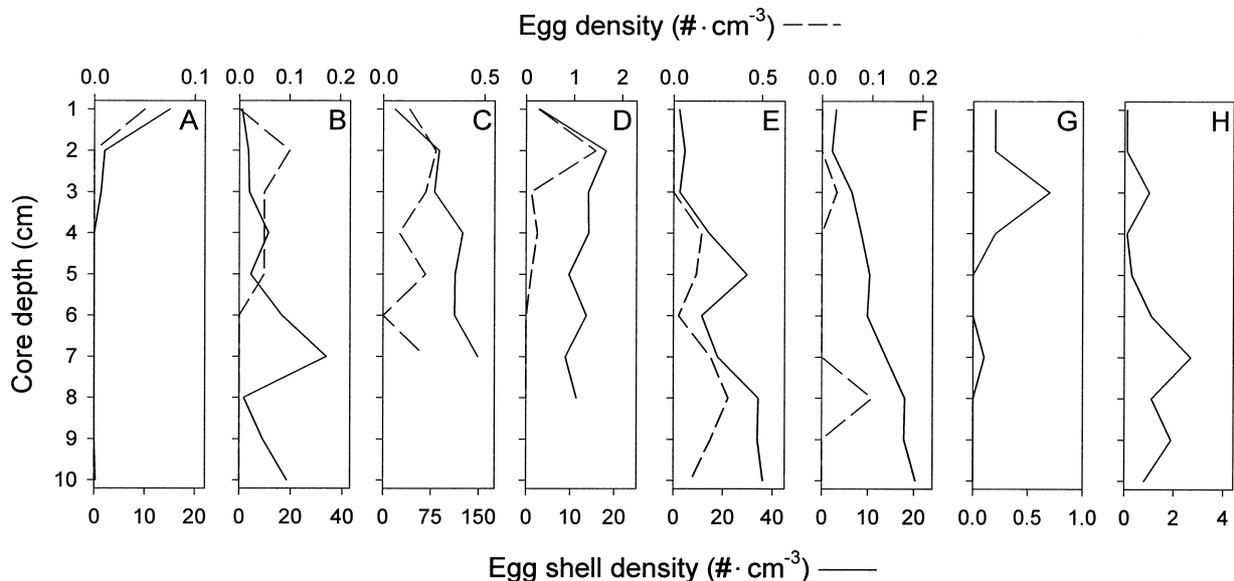


Figure 3. Density of viable diapausing eggs and egg shells in sediments of the eight study lakes. Four lakes have extant *Hesperodiaptomus* populations and lack fish (A–D), and four lakes currently lack *Hesperodiaptomus* populations and contain fish (E–H). Note that the two X-axes have different scales. The scale for egg density was left off of Figures G and H because these samples lacked any viable diapausing eggs.

layers described by Wood (1977:140–141, Figure 4, 5) as occurring in our study area and dating to 720 and 1200 yrs BP, respectively. The presence of egg shells in the 20–22 cm section, therefore, suggests that some *Hesperodiaptomus* egg shells persist in lake sediments for > 700 yrs.

Having shown that eggs shells in our sediment cores are from *Hesperodiaptomus* diapausing eggs, we now have an important tool that will allow us to reconstruct the historical presence of *Hesperodiaptomus* in Sierra Nevada lakes. This taxon plays an important role in structuring zooplankton communities of fishless alpine lakes throughout western North America (Anderson, 1980; Stoddard, 1987; Paul & Schindler, 1994), but has been eliminated from many lakes by fish introductions (Anderson, 1980; Stoddard, 1987; Knapp, 1996). In those lakes where introduced fish populations have disappeared, *Hesperodiaptomus* sometimes fails to reappear (Parker et al., 1996; Knapp and Sarnelle, unpublished data). For lakes in which *Hesperodiaptomus* has not reappeared, we are now using *Hesperodiaptomus* egg shells to determine whether *Hesperodiaptomus* was present prior to fish introductions. This information will allow us to identify those lakes in which *Hesperodiaptomus* reintroductions will be necessary to restore food web structure (McNaught et al., 1999).

If other copepod taxa produce eggs that leave recognizable shells behind after hatching (e.g., Parker et al., 1996), egg shells may have general utility in future paleoecological reconstructions of lake community structure. Their utility is likely to be greatest in lakes with a low diversity of zooplankton species, because this low diversity makes it easy to associate individual species with egg shells found in lake sediments.

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