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Boedeker, C.

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Global decline of and threats to *Aegagropila linnaei*, with special reference to the lake ball habit

Christian Boedeker, Anja Eggert,
Anne Immers & Erik Smets

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Abstract

The freshwater macroalga *Aegagropila linnaei* can occur attached or unattached, but is best known for its loose-lying spherical growth form ('lake balls', 'Cladophora balls', or 'Marimo'). The worldwide distribution of this species was reconstructed based on more than 1200 specimens from 28 herbaria, an extensive literature survey, and field observations. *Aegagropila linnaei* was found in 283 locations, with the majority located in central and northern Europe. Few records of *A. linnaei* exist from North America, but it is relatively widespread in Japan. The preferred habitat is inferred to be oligo-mesotrophic lakes with moderate to high calcium levels. In more than 50% of the known locations, *A. linnaei* has not been found in the last 30 years, and this decline is assumed to be correlated with changes in trophic conditions in those lakes. The unattached growth forms show a stronger decline than attached populations as a result of the habitat types in which they can occur.

Introduction

The freshwater green alga *Aegagropila linnaei* (Cladophorales, Ulvophyceae) is best known for its unattached spherical growth form, also referred to as “Marimo”, “lake balls”, or “*Cladophora* balls” (Fig. 1a). These lake balls can develop under certain hydrographic and topographic conditions either directly from the attached growth form (Fig. 1b), from floating unattached mats, or from fragmented older balls (Brand 1902, Kurogi 1980). Individual filaments of *A. linnaei* have a simple morphology of uniseriate branched filaments similar to *Cladophora* species (Fig. 1c). The filaments can be attached by a holdfast or secondary rhizoids to the substratum, forming low turfs on submerged rocks or bivalves, or can be intermingled and free floating as mats or balls (van den Hoek 1963, Leliaert & Boedeker 2007). The lake balls are more or less radially organized, and increase in size by the vegetative growth of the interwoven filaments. The stiff texture of the filaments and the production of secondary rhizoids are features that facilitate entanglement and subsequent ball formation. Water motion is the main shape-giving factor, and balls occur typically in shallow lakes with sandy bottoms. Rolling movements on the sediment and abrasion of apical cells induce radial growth and profuse lateral branching (Acton 1916). Different growth forms can co-occur under different conditions in the same lake (Luther 1951, Kurogi 1980).

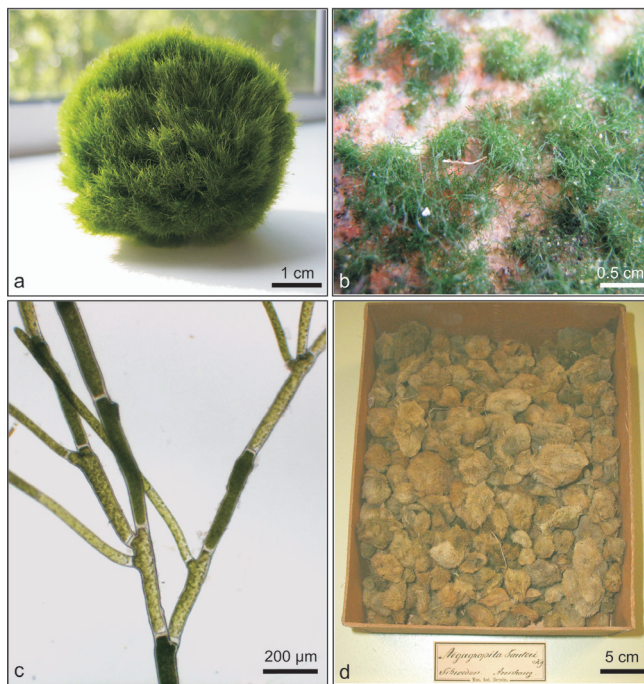


Figure 1. (a) Lake ball, ordered from an online aquarium shop. (b) Attached growth form of *A. linnaei* from Lake Erken, Sweden (photo: Roland Bengtsson). (c) Microscopical characters of *A. linnaei* filaments with typical subterminal and serial insertion of branches. (d) Herbarium box (in Botanical Garden and Museum Berlin-Dahlem, Germany) with old specimens (as *Aegagropila sauteri*) from a lake in Sweden.

The spherical growth form has attained considerable popularity. *Aegagropila linnaei* has been given the status of a “special natural monument” in Japan (Kurogi 1980), and the lake balls are shown on postage stamps from Japan and Iceland. In Japan, a whole range of “Marimo merchandise” exists, ranging from souvenirs and lucky charms with live specimens to toys and cartoons. *Aegagropila linnaei* balls have also become extremely popular in the aquarium trade in recent years.

In contrast to most other freshwater algae, *A. linnaei* has a restricted distribution, and is found mainly in previously glaciated areas of Europe. In addition, *A. linnaei* has been identified in a range of locations in Japan, whereas

only few records are known from North America. In most countries it is regarded as a rare species. Freshwater algae that are not widespread are often restricted in their distribution by specific ecological demand, such as desmids (Coesel *et al.* 1978) or macroscopic *Nostoc* species (Mollenhauer *et al.* 1999). On the other hand, a restricted distribution can also be the result of a limited dispersal capacity. Migrating birds are one of the most important dispersal vectors for freshwater algae (Schlichting 1960, Green *et al.* 2002), transporting desiccation-resistant stages such as akinetes. However, akinetes are basically unknown in *A. linnaei* (Brand 1902, Acton 1916, van den Hoek 1963). It can be speculated that limited dispersal abilities and competition effects have shaped the current distribution of *A. linnaei* more than has the availability of habitats.

Even though the species was initially assumed to occur mainly in eutrophic waters (van den Hoek 1963), eutrophication is mentioned as a threat in several more recent studies reporting declining populations of *A. linnaei*. Partly because of the popularity of the intriguing ball form, this is one of the few freshwater algal species that has been listed threatened on several red lists of endangered species. *Aegagropila linnaei* is included in the national red lists of Belarus (MINPRIRODA 2006), Estonia (Lilleleht 1998), Germany (Ludwig & Schnittler 1996), Japan (EAJ 2000), Russia (Noskov 2000), and Sweden (Gärdenfors 2005). In addition, *A. linnaei* is explicitly mentioned in the Ramsar specifications of Lake Akan, Japan (Moriwake 2005), and Lake Myvatn, Iceland (Gíslason 1992). In the United Kingdom, *A. linnaei* is included the list of rare algae and in the assessments of Important Plant Areas by Plantlife (Brodie *et al.* 2008).

For several locations, this species is already assumed or known to be extinct. In four out of the five locations in The Netherlands where *Aegagropila linnaei* was found 60 years ago, it is now absent, and the unattached forms seem to have disappeared altogether as a result of the effects of eutrophication (Boedeker & Immers 2009). Balls of *A. linnaei* have been extinct in Lake Zeller in Austria since around 1910, most likely due to effects of human activity (Nakazawa 1974), and only the attached filamentous form has been reported since then (Kann & Sauer 1982). The attached growth form is still present in several larger rivers of northern England and Scotland, but the ball-shaped growth form is found only in a few unchanged locations (John 2002). There is only one location in Denmark (Sorø Sø, Sjælland) where *A. linnaei* occurs, although the species had been found previously in more locations (van den Hoek 1963). In Lake Galenbecker, in northeastern Germany, carp cultivation and intensified agriculture in the 1960s led to eutrophication and extinction of the species (Pankow 1985). Also, in Japan, where the species is still relatively widespread, human activities have led to the destruction of many of *A. linnaei*'s natural habitats. Populations of *A. linnaei* have disappeared from two bays in Lake Akan, most likely as a result of increased mud deposition (Wakana *et al.* 2006). Population declines (both in population density and distribution area) have been monitored in the Takkobu Marsh, Japan, between 1996 and 2004, and are probably a result of the effects of eutrophication, such as higher nutrient load, accumulating layers of mud and silt on the marsh bottom, shading by phytoplankton blooms, and an increase in the depth limit of freshwater bivalves (Wakana *et al.* 2005). Declining populations have also been observed in several other small swamp lakes for the same reasons (Wakana *et al.* 2001b). The *Red Data Book of Estonia* lists eutrophication, changes in water hydrology, and dredging as factors that threaten the survival of *A. linnaei* (Lilleleht 1998).

In this study, detailed information on the distribution of *A. linnaei* based on herbarium specimens (Fig. 1d), literature data, and field observations is used to assess whether there is a global trend of declining populations. Habitat preferences with regard to trophic level are inferred, and we discuss possible deleterious effects of eutrophication. Furthermore, we evaluated whether unattached populations, including the ball form, are more threatened than the attached growth form.

Materials and Methods

Location survey

We surveyed herbarium collections to produce detailed distribution maps of *A. linnaei*. Fifty-nine herbaria responded to our inquiries, of which 28 had collections of *A. linnaei* (for a list of *A. linnaei* synonyms, see Appendix S1 in the Supplementary Materials; see Appendix S2 for a complete list of herbaria). We borrowed from or visited collections, and identified the samples using a light microscope (Olympus BH2) after rehydrating fragments in water. In total, we checked approximately 1200 herbarium specimens of *A. linnaei*, about half of which had location details, and a third of which (about 400 specimens) had a collection date (Table 1). Almost all specimens with missing information seem to be about a century old, judging from the general appearance of the vouchers and the handwriting. In addition to the location and collection date, we recorded the growth form wherever possible. The resulting data set was amended by an extensive literature survey and direct inquiries with local and national water-monitoring organizations or with limnological departments in countries where *A. linnaei* was known or suspected to occur. These inquiries were particularly useful in obtaining information on the existence of extant populations and on past and present trophic levels of the habitats. We reduced this data set to a digest listing all locations (see Appendix S3), and georeferenced the locations. Excluded specimens, locations, and synonyms are listed in Appendices S1 and S3. We created maps using Manifold GIS (geographic information system) software, and displayed data using the World Geodetic 1984 projection. We treated multiple records from the same lake, that is, in different years or in different areas of the same lake, as one location. Almost one-fifth of the locations are from the Baltic Sea, so these locations were grouped together as Baltic proper, western Gulf of Bothnia, eastern Gulf of Bothnia, Gulf of Finland, and Bay of Bothnia, each counting as one location to deflate total location numbers, since individual lakes represent locations in freshwater environments. Records younger than 30 years have been classified as “extant populations” and are listed separately in Appendix S4.

Origin of Aegagropila linnaei balls in the aquarium trade

We contacted more than 20 aquatic plant traders in Belgium, Denmark, Germany, Japan, the Netherlands, Singapore, Ukraine, and the United States to find out about the origin of *A. linnaei* balls for sale in commercial aquarium shops, and to learn about the possible supply from wild stocks.

Table 1. Number of herbarium specimens, locations, and extant populations of *Aegagropila linnaei*.

Herbarium specimens	Number
Herbarium specimens	1200
Herbarium specimens with recorded locations	661
Herbarium specimens with recorded dates	404
“Ball-shaped” herbarium specimens	310
Locations	
Locations (including literature and field observations)	283
Locations corrected for Baltic Sea ^a	233
Locations with known growth forms ^a	185
Locations with attached growth form ^a	61
Locations with unattached growth form (ball-shaped) ^a	124 (90)
Extant locations (< 30 years)	
Extant locations ^a (percent decline)	101 (57%)
Extant locations with attached growth form ^a (percent decline)	33 (46%)
Extant locations ^a with unattached/ball-shaped form (percent decline)	51/36 (60%)
Top six countries: no. locations (no. extant locations)	
Sweden ^a	51 (23)
Germany	33 (8)
Great Britain and Ireland	32 (16)
Japan	21 (20)
Russia ^a	19 (2)
Iceland	10 (9)

^aBaltic Sea is divided into five areas: Baltic proper, Gulf of Bothnia West, Gulf of Bothnia East, Gulf of Finland, Bay of Bothnia, each counting as one location.

Results

Location survey

Table 1 shows an overview of the herbarium and literature survey. In total, *A. linnaei* had been recorded from 283 locations (Appendix S3), or rather 233 locations when the list is corrected for the continuous coastline of the Baltic Sea. We sorted the dated herbarium specimens of *A. linnaei* in intervals of 25 years to illustrate the distribution of collections over time (Figs. 2a, b, c). The first two time intervals in each diagram are underrepresented, since about 600 herbarium specimens did not have a date but were clearly very old, as indicated by the dashed lines. Both the number of specimens (Fig. 2a) and their locations (Figs. 2b, c) show a decrease since the beginning of the last century. This decrease is most pronounced from 1900 onward, and again from 1975 onward. The number of locations represented by herbarium specimens dropped from 32 (1875–1899) to 7 (1975–2007), representing a decline in locations of 78%. The number of locations for ball-shaped specimens dropped from 27 to 5 in the same time interval (Figs. 2b, c). Since herbarium collections reflect not only the occurrence of species at a particular time and place but also the degree of collecting activity, we complemented the herbarium data set with a comprehensive literature survey and field observations, which yielded an additional 152 locations (see Appendix S3 combined data shown in Fig. 2d).

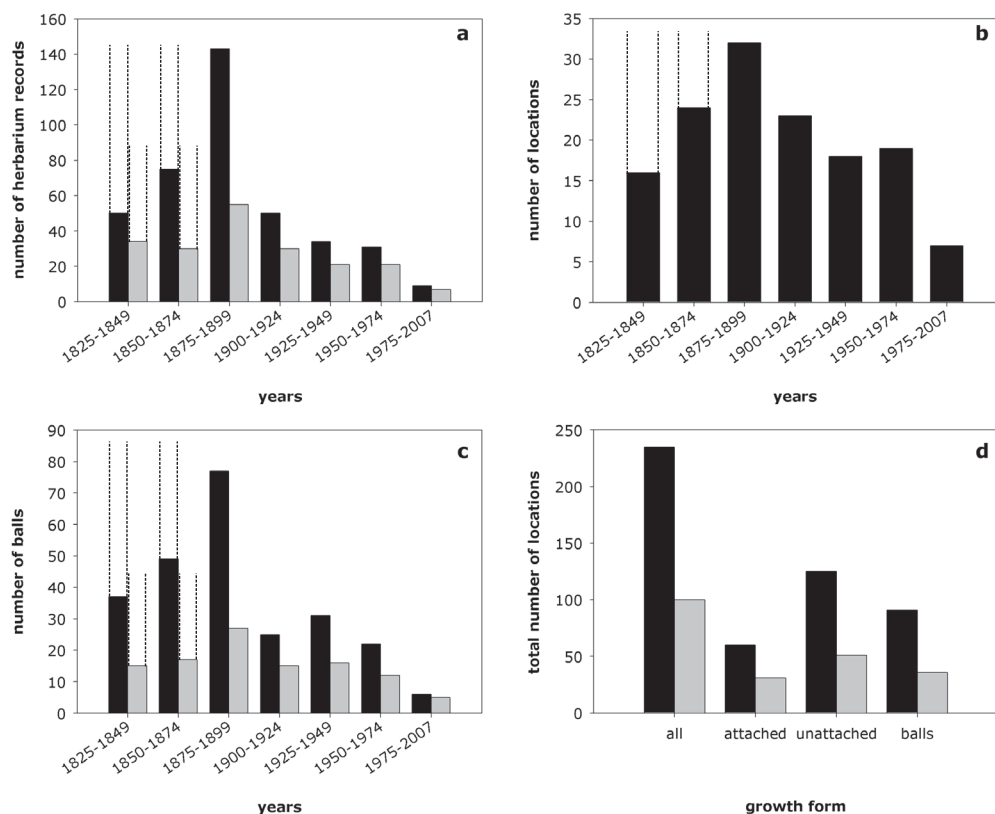


Figure 2. (a) Number of herbarium specimens of *A. linnaei* over time. Black bars indicate the total number of herbarium specimens, gray bars indicate the number of herbarium specimens corrected for duplicates. (b) Number of locations of *A. linnaei* over time based on herbarium specimens. (c) Number of herbarium specimens (black bars) and locations based on herbarium specimens (gray bars) of the ball-shaped growth form of *A. linnaei*. (d) Total number of known locations of *A. linnaei* (black bars) and known extant populations (< 30 years; gray bars), based on herbarium specimens, literature, and field data. Dashed lines indicate the large number of very old herbarium specimens that had no date.

We classified records from less than 30 years ago as “extant populations,” and these are listed with the most recent collection date in Appendix S4. *Aegagropila linnaei* has been found in 107 locations since 1976, or 101 locations when correcting this number for multiple locations within the same area of the Baltic Sea (Table 1; Appendix S4). Of the 235 total locations where *A. linnaei* had been found originally, it has not been recorded in the last 30 years in about 57% of those locations (Fig. 2d). All extant populations are indicated in Fig. 3. Outside Europe, extant populations of *A. linnaei* can be found in a considerable number of locations in both Iceland and Japan (Fig. 3a). Within Europe, the majority of extant populations exist in Sweden, Great Britain, Ireland, and northeastern Germany (Fig. 3b) - thus in areas that also had the highest density of original habitats. In the Alps, one of the clusters of original habitats, *A. linnaei* has been reported from only a single location in the last 30 years (Lake Zeller). For the majority of European locations, no recent findings of *A. linnaei* have been made.

Differences in *A. linnaei* population declines have been found for the different

growth forms (Fig. 2d). The presumed loss of locations is less pronounced for the attached growth form (48% decline) than for the unattached forms (59% decline; ball form only: 60% decline).

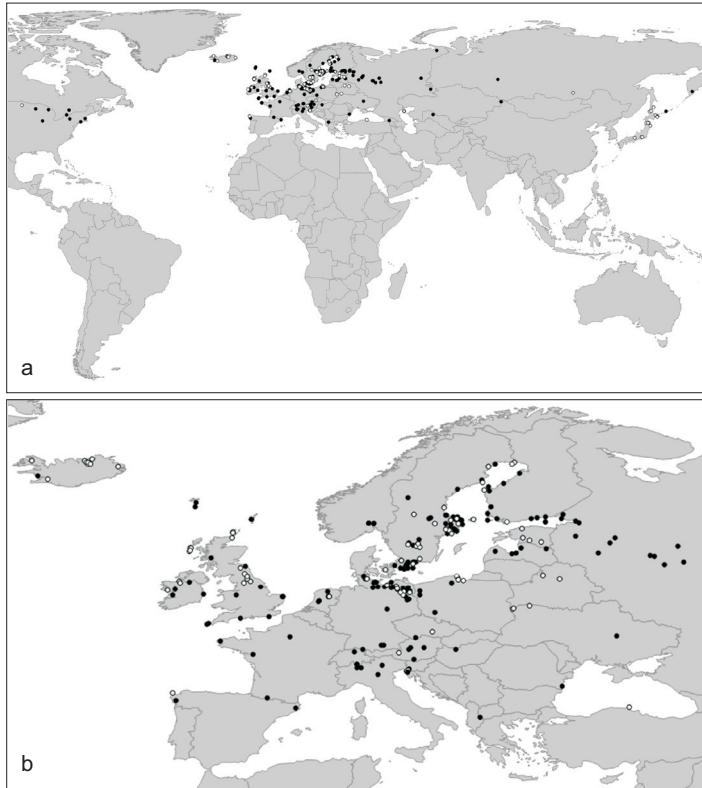


Figure 3. (a) All known locations of *A. linnaei* (black dots) and extant populations (< 30 years; white dots). (b) Shows a more detailed map of Europe.

A direct comparison of original and extant European locations in relation to the growth forms of *A. linnaei* is given in Fig. 4. Figs. 4a and 4b show all known attached populations (historical and current) and the extant attached populations, respectively. For about 48% of the locations (corrected) with the attached growth form, *A. linnaei* has not been reported in the last 30 years. In Figs. 4c and 4d, both the original and the extant unattached populations are compared, further broken down into floating and ball-forming populations. Unattached growth forms have been found more commonly in Europe than the attached growth form (Figs. 4a, 4c). However, when comparing the number of extant ball-forming populations with the number of historical habitats for this growth form, for about 60% of those locations *A. linnaei* has not been reported in the last 30 years (59% for all unattached growth forms combined).

Origin of A. linnaei balls in the aquarium trade

Of more than 20 contacted aquarium shops and aquatic plant trading companies, 13 were willing to share at least some information concerning the origin of the “lake balls” in their product line. Generally, companies were very reluctant to reveal their suppliers, growers, or wholesalers. However, in all instances where information was obtained,

Ukraine was given as the country of origin, sometimes specified as Lake Svityaz, or the Shatsk lakes, in northwestern Ukraine. Local Ukrainian sellers of *A. linnaei* balls in Odessa and Kyiv said that they receive their material periodically from Lake Svityaz, but claimed that they grow the majority of balls themselves. Most companies grow balls vegetatively from fragments to market size, but receive stocks from Ukraine. Balls from Lake Svityaz are shipped even to aquatic plant-breeding facilities in Southeast Asia before they are returned to the European market. Japanese aquarium shops sell only balls of European origin. No other natural source of *A. linnaei* balls other than the Shatsk lakes (including Lake Svityaz) was ever mentioned by people in the aquarium plant-trade business.

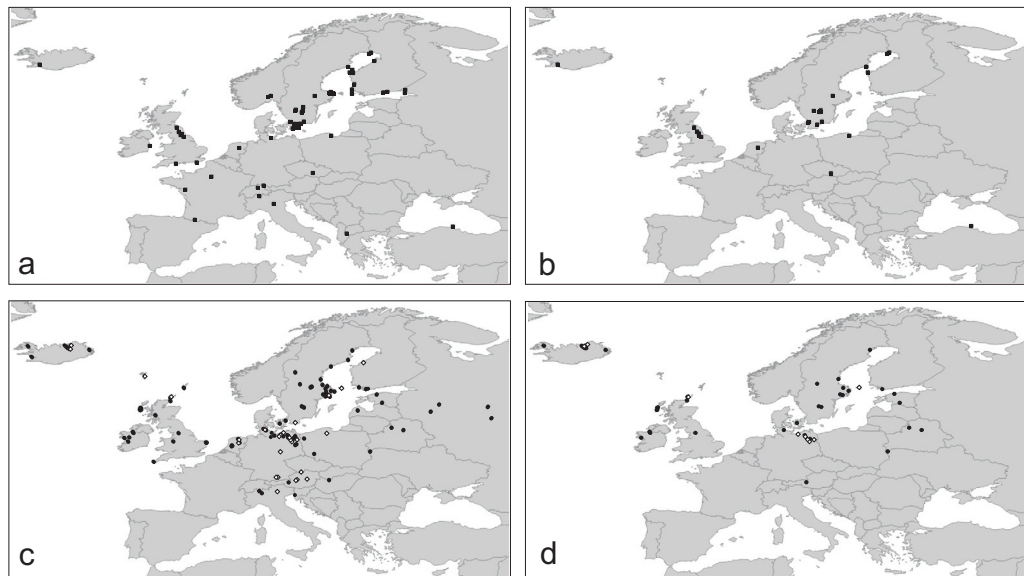


Figure 4. Direct comparisons of all known and extant locations of *A. linnaei* in Europe, shown separately for attached and unattached growth forms. (a) All known locations of attached populations. (b) Extant locations of attached populations. (c) All known locations of unattached growth forms (black dots: unattached filaments/mats, white dots: balls). (d) Extant locations of unattached growth forms (black dots: unattached filaments/mats, white dots: balls).

Discussion

Evidence for decline of A. linnaei populations

Detailed knowledge of the distribution of a species is a prerequisite for (a) assessing the status of that species, (b) monitoring (a decline of) its populations, (c) identifying general threats, and (d) employing most conservation efforts. Typical difficulties in reconstructing the area of distribution are related to incomplete or inaccessible records, patchy local knowledge, and to verifying observations made by third parties. Many organisms, especially among the algae, must be regarded as underrecorded, and are frequently misidentified. This is most likely also the case with *A. linnaei*, despite all efforts to assemble as complete a data set as possible. Even though this species has for a long time intrigued scientists (e.g., Brand 1902, Wesenberg-Lund 1903, Acton 1916, Kindle 1934, Waern 1952), very few reports have provided anything more than scanty lists of findings (Palik 1963, van

den Hoek 1963, Pankow 1965, Getzen 1967). All biogeographic reconstructions are hampered by incomplete distribution records, and for most algal species, the knowledge of the global distribution is poor and based on many assumptions. Although some regions of the world are well sampled, such as (western) Europe, the largest parts of the globe are not. The absence of *A. linnaei* in northern North America and Siberia (Fig. 3a) could be an artifact of undersampling, despite our efforts to locate records from those areas. Even though it seems unlikely that *A. linnaei* would occur in South America, suitable habitats are potentially abundant in the Andes region, but nobody has looked.

The studies on the extinction or decline of *A. linnaei* cited above are all restricted to a confined area, mostly one individual location. We investigated whether a general trend of declining populations could be revealed by analyzing as many historical and recent findings as possible. Nevertheless, our record of *A. linnaei* is most likely incomplete, despite the fact that the occurrence of the enigmatic balls in a lake is frequently recorded or commented upon, as illustrated by a wealth of historical scientific and amateur literature. Thus, we assume underrecording is less of a problem for *A. linnaei* than for most other algal species, at least for its unattached growth forms. Even though the observed decline in herbarium specimens does probably also reflect a decline in the submission of voucher specimens, the herbarium collections show the same declining trend as the more comprehensive survey, which also includes data from the literature and communicated field observations (Figs. 2d, 3). Extant populations are known for less than half of the original locations (see Fig. 3a). The inferred decline might be affected by another methodological problem, that is, for some regions (e.g., Russia), old literature is available but no or few personal contacts exist, and recent literature is difficult to obtain (see Table 1).

Differences in the degree of decline were found between attached and unattached growth forms, with the loss of unattached forms being more pronounced (Fig. 2d). Unattached forms usually float just above the sediment, and therefore occur only in shallow lakes or shallow parts of a deeper lake, where the euphotic zone reaches the bottom. It seems that the stronger decline of unattached growth forms is not caused by a higher sensitivity to environmental changes compared with the attached forms, but rather by a higher threat to their shallow habitats. The attached form most commonly occurs on submerged rocks or freshwater bivalves (Luther 1951, Waern 1952, Pankow & Bolbrinker 1984, Wakana *et al.* 2001b), and has been found in depths of 20 meters (m; maximum depth 50 m) in deep lakes (Brand 1902, Kindle 1915), as well as in a number of rivers (Holmes & Whitton 1975, 1977, 1981a, Gardavský 1986, Whitton *et al.* 1998). The attached form might be able to escape greater sedimentation and better cope with decreasing underwater light availability by colonizing substrates closer to the surface, and therefore increasing chances of survival in disturbed habitats. In addition, the ability to survive in deep and steep lakes increases the absolute number of potential habitats for the attached form, and those lakes are generally less sensitive to anthropogenic changes than shallow lowland lakes (e.g., Taranu & Gregory-Eaves 2008). The attached form is distributed continuously only in the low-salinity areas of the northern Baltic Sea (Fig. 2b), where species numbers and competition are low. *Aegagropila linnaei* forms dense carpets on rocks in the Bay and Gulf of Bothnia, and can be the dominant macroalga in areas with a salinity below 4 practical salinity units (Bergström & Bergström 1999). However, the number of recent records from that area indicates a declining population as well (Figs. 3, 4). The decline of unattached growth forms is most obvious in central Sweden, northeastern Germany, and the subalpine region of the European Alps (Figs. 4c, d).

Eutrophication as the main cause for the decline of Aegagropila linnaei

Human activities increases and accelerates the external supply of nutrients to aquatic ecosystems worldwide (e.g., Smith *et al.* 1999, MEA 2005). Freshwater habitats especially face increasing threats from physical alteration, changes in water level and salinity, overexploitation, introduction of nonnative species, herbicide and other biocide runoff, airborne pollution, and nutrient loading (MEA 2005, Revenga *et al.* 2005). Eutrophication of aquatic ecosystems is a common process worldwide and leads to the loss of unique habitats and a reduction in biodiversity (e.g., Bayly & Williams 1973, Smith *et al.* 1999).

Enrichment of nitrogen and phosphorus loadings selects for fast-growing algae (phytoplankton, macroalgae such as *Ulva* or *Cladophora* species) at the expense of slower-growing species (Duarte 1995), such as some charophytes, for example, or *A. linnaei*. Shallow lakes are especially prone to the regime shift from aquatic macrophytes to phytoplankton dominance. The decline of several freshwater algal groups has been attributed to eutrophication effects; such groups include macroscopic *Nostoc* species (Mollenhauer 1998, Mollenhauer *et al.* 1999), desmids (Coesel *et al.* 1978, Geissler 1988), and charophytes (Geissler 1988, Blindow 1992, Nagasaka *et al.* 2002). A number of reports on the local decline or extinction of populations of *A. linnaei* mention eutrophication as a responsible factor (Pankow 1985, Wakana *et al.* 2001b, 2005, 2006, Boedeker & Immers 2009).

Table 2. Trophic conditions of lakes where *Aegagropila linnaei* occurred historically or still occurs.

Lakes with information available on the pristine state (n=74)	Percentage
Oligotrophic (including dystrophic)	37%
Oligo-mesotrophic	8%
Mesotrophic	47%
Slightly eutrophic	7%
Eutrophic	1%
Lakes with information available on both the pristine and current state (n=61)^a	
Pristine state oligotrophic (including dystrophic and oligo-mesotrophic)	39%
Pristine state mesotrophic	53%
Pristine state eutrophic (including slightly eutrophic)	8%
Current state oligotrophic	20%
Current state mesotrophic	23%
Current eutrophic	57%
Lakes with information available on the current state (n=80)^a	
Current state oligotrophic	16%
Current state mesotrophic	18%
Current eutrophic	66%
<i>Aegagropila linnae</i> still present	39%

^aIf a lake had a history of eutrophication but has been restored to a lower nutrient load it is filed under the most severe stage of eutrophication in its history.

Our literature study showed that *A. linnaei* occurs in several different lake types, but most typically in shallow, oligo-mesotrophic, glaciofluvial lakes with reed stands, dense charophyte vegetation, a pH greater than 7, and moderate to high calcium levels. However, the ecological preferences or requirements of *A. linnaei* had never been fully characterized, and conflicting views can be found in the literature with regard to nutrient levels. In a classic volume on European *Cladophora* species, it is stated that *A. linnaei* (as *Cladophora aegagropila*) “seems to occur only in more or less eutrophic water” (van den Hoek 1963). In this study, we collected information on trophic level and changes therein for a considerable number of lakes where *A. linnaei* occurred historically or still occurs (summarized in Table 2). For 74 lakes, information on the pristine state (*i.e.*, the trophic level of a water body without anthropogenic influences) was available (Table 2). Ninety-two percent of these lakes were either oligo- or mesotrophic in their pristine state, indicating the typical habitats of *A. linnaei* with regard to the trophic level that contrast with van den Hoek’s statement (1963). For 61 lakes, information on both the pristine state and the current trophic level could be compared. Of these lakes, 92% were originally oligo- or mesotrophic, but more than half of those 61 lakes (57%) are currently eutrophic, thus dramatic changes have occurred in many habitats of *A. linnaei* in the recent past. When looking at all lakes with information on the current trophic state ($n = 80$), the numbers are even more dramatic. Sixty-six percent of the lakes are now eutrophic, or had been in the recent past, and *A. linnaei* still occurs in only 39% of those 80 lakes. These numbers correspond to the general findings presented in Fig. 2, and strongly suggest that *A. linnaei* occurs mainly in oligo-mesotrophic habitats and that eutrophication is correlated with the observed decline of *A. linnaei* populations.

Seemingly contrary to this inferred habitat preference, extant populations of *A. linnaei* have been found in 15 eutrophic locations (each addressed below). However, in five of these locations (Takkobu marsh, Pon swamp, Kimoma swamp, Lake Akan, and Boven Wijde; see Appendices S3 and S4) a strong decline in population size has been observed (Wakana *et al.* 2001b, 2005, 2006, Boedeker & Immers 2009, respectively), and the future of these populations must be regarded as uncertain. Generally, most eutrophic lakes are turbid and have a poor underwater light climate, but eutrophic, clear-water lakes also exist. Shallow lakes, with abundant submerged macrophyte vegetation, may have very clear water with sparse phytoplankton despite relatively high nutrient loadings in lowland areas with soft rock (Phillips *et al.* 1978). Biological interactions in clearwater lakes differ markedly from “regular” eutrophic lakes (*e.g.*, Jeppesen *et al.* 1999). The eutrophic clearwater lake Mývatn (Iceland) is well known for its population of *A. linnaei* balls (Einarsson *et al.* 2004). In Germany, *A. linnaei* has been found in a couple of eutrophic clear-water lakes (Neuklostersee and Teterower See). In Lake Biwa and Lake Kawaguchi (both in Japan), only restricted parts of the lake are eutrophic, while large parts are still mesotrophic (Nagasaka *et al.* 2002). Two brackish locations of *A. linnaei* are classified as (slightly) eutrophic (Pojo Bay, Finland, and Lake Mälaren, Sweden), as well as one river (River Wear, Scotland). The only “regular” eutrophic lakes where *A. linnaei* was found are Lake Ülemiste (Estonia) and Lake Tiefwareensee (Germany), but the latter had been recently restored to mesotrophic conditions. Even though the evidence is correlative, these numbers strongly suggest that *A. linnaei* occurs mainly in oligo-mesotrophic habitats but can persist in eutrophic, clear-water lakes, and that indirect effects of eutrophication caused the observed loss of *A. linnaei* populations.

The possible role of lake acidification in the decline of Aegagropila linnaei

Other factors besides eutrophication may play a role in the decline of *A. linnaei*, such as the widespread and serious problem of lake acidification. Acidification is mainly caused by the transfer of airborne pollutants by wind from heavily industrialized centers to distant areas where they precipitate as acid rain. Especially in areas where lakes have a low buffering capacity, the effects of acid rain can be drastic, with pH values dropping below 5. Acidification can be a primary and serious problem resulting from airborne dispersal of acidifying pollutants in habitats in remote areas that are not affected by agricultural eutrophication. Many lakes in eastern North America, Great Britain and Ireland, and Scandinavia (except southern Sweden) are characterized by low calcium values and thus a low buffering capacity, which makes them very sensitive to acidification (Jørgensen 1997, Arts 2002). The vegetation of those softwater lakes is highly endangered, and 90% of the habitats of softwater vegetation in the Atlantic region have already disappeared (Brouwer *et al.* 2002). Typical softwater environments are oligotrophic, with a pH between 5.5 and 7, and have a low buffering capacity (Arts 2002). However, *A. linnaei* is not a typical softwater organism; instead, the vast majority of habitats are well buffered and characterized by moderate to high calcium levels and an alkaline pH between 7 and 9. No records exist from known acidic habitats. Generally, even less information is available on (historical) acidity than on trophic levels. In the course of this study, only five habitats of *A. linnaei* were found with a pH below 7 (the lowest known pH is 6.3; historical data for Lake Bültsee, Germany), and only eight lakes were reported to have low calcium levels. This indicates that *A. linnaei* typically occurs in habitats that are not strongly affected by acidification. Furthermore, most lakes in Scandinavia have naturally low conductivity, low calcium content, and low pH (Henriksen *et al.* 1988, Jørgensen 1997), which could possibly explain the absence of *A. linnaei* in Norway and Finland (Fig. 3b).

Acidification has been shown to be a serious problem in remote mountain lakes that are otherwise unaffected by direct pollution (Curtis *et al.* 2005). Although several lakes in the Alps region in which *A. linnaei* was historically found were clearly subject to eutrophication (e.g., Ammersee, Lago di Como, Lago Maggiore, Lago di Varese, Starnberger See), it cannot be excluded that acidification might pose an additional threat in more remote mountain areas in noncalcareous parts of the Alps, the Pyrenees, or central Asia. Likewise, acidification might negatively affect *A. linnaei* populations in otherwise unpolluted lakes elsewhere, and thus pose an additional threat of eutrophication in some regions. However, no conclusive data are currently available.

Which effects of eutrophication might cause the observed decline?

Competitive exclusion in the face of increased nutrient loading appears to be the main mechanism responsible for the local decline or even extinction of species in temperate freshwater ecosystems (Barker *et al.* 2008); that is, species better adapted to high trophic levels outcompete others when nutrient loads increase. It had been proposed that high levels of phosphorus have a toxic effect on charophytes (Forsberg 1965), but this was not confirmed in other studies (Blindow 1992). Even though direct deleterious effects of high nutrient availability are discussed in terrestrial ecosystems, changes in the light climate of aquatic systems seem to be the most important (Smith *et al.* 1999). The finding of *A. linnaei* in several eutrophic clearwater lakes (see above) could be an indicator

that the increase of nutrient levels itself does not negatively affect *A. linnaei*. Shading by increased phytoplankton densities or by opportunistic microalgal epiphytes might be especially significant for *A. linnaei* as a very slow-growing species (Acton 1916, van den Hoek 1963). However, this species survives relatively long and dark winters under the ice in high-latitude lakes such as in Iceland (Jónnson 1992, Einarsson *et al.* 2004), and healthy balls of *A. linnaei* were found below the shallow photic zone in Lake Teterow, Germany (Pankow & Bolbrinker 1984). This illustrates the capacity of this species to cope with low-light conditions, suggesting that other factors than light limitation might play a more important role in the decline of *A. linnaei* populations.

The attached growth form has been found in several eutrophicated environments such as the Seine in Paris (van den Hoek 1963), Lake Boven Wijde in the Netherlands (Boedeker & Immers 2009), and several Japanese swamp lakes (Wakana *et al.* 2001b, 2005). It is possible for the attached form to survive if it grows on rocks or bivalves close enough to the surface to still receive sufficient light while the water body becomes more turbid. In Lake Myvatn in Iceland, it has been proposed that variation in the size of submerged *A. linnaei* mats is negatively correlated with (shading) blooms of the cyanobacterium *Anabaena flos-aquae* (Einarsson *et al.* 2004). Toxins released by blooming cyanobacteria could also be responsible for or contribute to the decline of *A. linnaei* in eutrophicated lakes. Other phenomena correlated with eutrophication that could be linked to the decline of *A. linnaei* are (a) increased feeding of herbivorous fish such as bream, carp, and roach (ten Winkel and Meulemans 1984); (b) local benthic anoxia; (c) higher levels of herbicides and pesticides; (d) greater organic sedimentation; and (e) increased mechanical damage and turbidity caused by recreational boats.

Threat assessment and conservation

Although the exact effects of eutrophication that cause a decline in populations of *A. linnaei* are unclear and require further experimental studies, it seems evident that the decline is correlated with eutrophication. This of course is a general problem that is not easily solved, since the process is closely linked to agriculture, tourism, and human demand and population growth, and is therefore linked to politics and economies. In addition, the populations of *A. linnaei* in several regions and habitats may face specific local problems; for instance, the acid rain degradation of unpolluted, remote habitats. The most famous location of lake balls, Lake Akan, in Japan, is struggling not only with eutrophication and general pollution but also the collection of *Aegagropila* balls as souvenirs. This habit had become so popular that in 1977 the species was classified as critically endangered, and the government asked people to return collected balls to the lake (Soejima *et al.* 2008, TMLA 2009). Since the populations from the Shatsk lakes in Ukraine seem to be the sole source of the popular *A. linnaei* balls for the entire aquarium trade, these populations must be under considerable, if not extreme, harvesting pressure, especially considering the slow growth rates and longevity of the species (see van den Hoek 1963). In this context it is interesting to note that a Canadian study on nonindigenous plants showed *A. linnaei* to be one of the most common species in the Saint Lawrence Seaway (Cohen *et al.* 2007), most likely anthropogenically introduced balls originating from amateur aquaria.

Very few molecular studies on *A. linnaei* exist to date. Recently, it has been shown that Lake Akan contains most of the genetic diversity of *A. linnaei* in Japan, as inferred by isozyme studies; therefore Lake Akan is the most important target of conservation

efforts (Soejima *et al.* 2008). Loss of genetic diversity might have occurred already through habitat degradation and extinction of local populations, and further reduction in genetic diversity is to be expected. In many areas, formerly eutrophicated water bodies have been restored to more-or-less natural conditions (van Liere & Gulati 1992, de Bernardo & Giussani 1995). Even after restoration, ecosystems do not necessarily return to their original diversity and community structure (Entwisle 1997). Because of the absence of desiccation-resistant stages in *A. linnaei*, in combination with the species' slow growth and the rarity of spore formation (Leliaert & Boedeker 2007), this species must be regarded as a poor disperser and a poor competitor. Therefore, recolonization of restored habitats might not be very likely. Once a habitat is successfully recolonized by *A. linnaei*, it might take decades before populations are large enough to develop into the typical unattached mats or lake balls. Even if a restored habitat were recolonized, genetic diversity might still be lost. Most extant populations must be regarded as threatened by eutrophication, except in some remote locations. The widespread attached form of *A. linnaei* in the northern Baltic Sea seems to be a stable component of the local algal flora, even though eutrophication has also taken place in this habitat. It is possible that reduced competition in brackish environments plays a role in maintaining *A. linnaei* populations.

One hopes that the popularity of lake balls will help with the conservation of this species and raise awareness of threatened freshwater algae. In addition, we would like to encourage the collection and submission of specimens to accessible collections, not just of *A. linnaei*, but also of species that are less showy and that currently receive less attention.

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