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The role of fish in distribution and germination of seeds of the submerged macrophytes *Najas marina* L. and *Ruppia maritima* L.

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Summary. The effects of three species of fish (tilapia, grass carp and common carp) on the seeds of *Najas marina* L. and of *Ruppia maritima* L. were investigated. Practically all the seeds that were injested by the common carp were digested. The two other fish were less affective: they digested seeds with soft seed-coats but excreted a good portion of the hard ones. Germination of the excreted seeds was improved. Seeds have been retained in the digestion tracts of the fish for up to 65 h. It is thus suggested that tilapias and grass-carps play a role in the distribution and the improvement of reproduction of *Najas* and *Ruppia*.

Key words: *Najas marina* – *Ruppia maritima* – Fish – Germination – Distribution

It is well known that various types of seeds, with different qualities of seed-coats are produced on one plant or even on one inflorescence (Howe 1986). Water plants are not exception in this respect and different types of seeds exist among them: seeds with soft seed coats as well as seeds with hard ones (Forsberg 1965; Agami and Waisel 1984). Intermediate types were also found in *Najas marina*, even on one specimen. The frequent occurrence of such an important characteristic raises the question of whether it is of ecological meaning.

Seeds of aquatic plants are known to be distributed by various animals, including birds (Vlaming and Proctor 1968; Pijl 1972; Agami and Waisel 1986) and fish (Gottsberger 1978; Prejs 1984; Carpenter and Lodge 1986). How does the quality of the different seed-coats affect the life span of those seeds? and do such different types of seeds play a role in the distribution and establishment of submerged water plants?

Najas marina L. and *Ruppia maritima* L. are submerged macrophytes with a wide distribution in the warm and temperate regions of the world (Van Vierssen et al. 1984; Triest 1986). In Israel, both species are found in natural water bodies, in fish ponds and in artificial water reservoirs. In such places, *Ruppia maritima* tends to occupy the more saline water habitats, whereas *Najas marina* grows mainly in the less saline ones (cf. Broch 1982a, b; Agami et al. 1984).

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Fresh and uninjured seeds of the two submerged species exhibit scattered germination with very low rates (Agami and Waisel 1984; Van Vierssen et al. 1984). However, germination of seeds whose seed coats were mechanically cracked, increased considerably (Agami and Waisel 1984, 1986). Therefore, it was assumed that a certain agent, which ruptures the seed coats of these plants, must exist in nature. Such a mechanism would explain the rapid establishment of these species in new habitats (see also Viinikka et al. 1987; Triest et al. 1988).

Seeds of *Najas marina* and *Ruppia maritima*, as well as seeds of many other species of hydrophytes are eaten by migrating waterfowl (Martin et al. 1951). In a previous study we have shown that mallard ducks play a role in long-distance distribution of *Najas marina* and in the improvement of its germination capability (Agami and Waisel 1986). However, waterfowl are not the only agents which feed on seeds of aquatic macrophytes, disseminate them and affect their germinability. Fish, that share the habitats of *Najas marina* and *Ruppia maritima* are also known to feed on such plants (Terrell 1975; Mitzner 1978; Leventer 1984). Therefore, in the following investigation, we have studied the effects of three species of fish on the seeds digestibility, on the retention time of such seeds in the intestines of the fish and on the after-effects of such passage on the excreted seeds. Attempts were made to correlate such parameters with the structural and mechanical qualities of the seed coats of the various types of seeds of *Najas marina*.

Materials and methods

Seeds of *Najas marina* L. were collected in June 1984 in an artificial water reservoir at Merkaz Sappir, Israel, in the Rift Valley some 45 km south of the Dead Sea (30°17'N; 35°11'E). The seeds were stored dry and kept in the dark, at room temperature. Most seeds of *Najas* have hard and dark seed coats. A few seeds are soft with either green or brown colour. An intermediate type of full-size but light-colored seeds was also observed.

Seeds of *Ruppia maritima* were collected in June 1986 in a fish pond in Ma'agan Mikhael in the coastal plain of Israel (32°33'N; 34°54'E). The seeds were stored dry in the dark at room temperature. All the seeds of *Ruppia* were of one type and had hard and dark seed coats.

Five days before experimentation the seeds were soaked in tap water at room temperature. On the day of experimentation, intact seeds were mixed with a standard fish food,

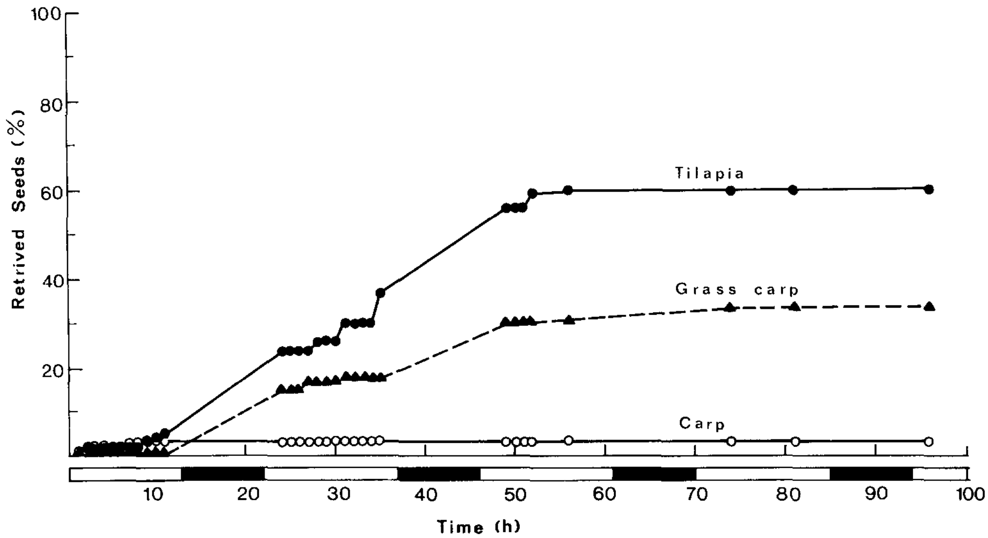


Fig. 1. Time course of seed-retrieval from the digestion tract of hungry fish. One hundred mixed seeds of *Najas marina* were ingested by each of the three fish species. Day and night hours are marked on the abscissa

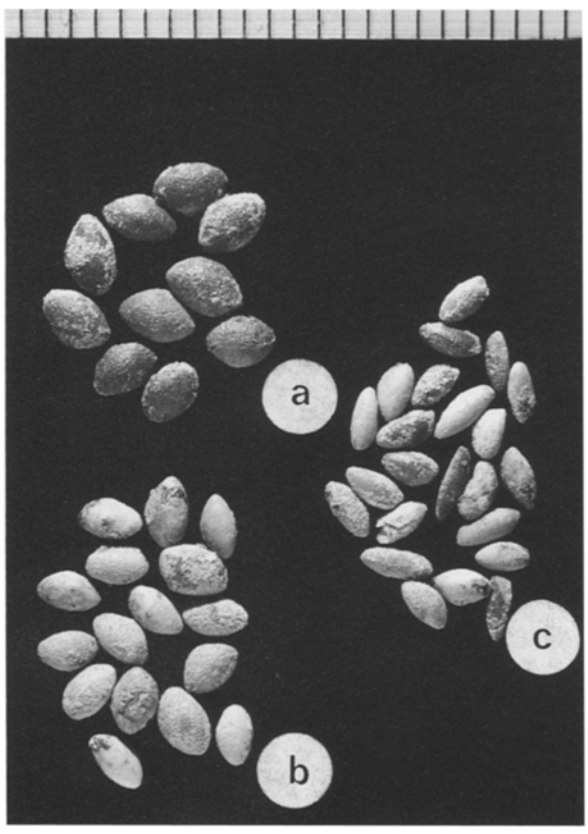


Fig. 2. Seeds of *Najas marina*: **a** – hard seeds, **b** – light-coloured seeds, **c** – soft seeds. Scale in mm

made into pellets and fed to the fish. Care was taken that all the pellets were eaten. Following feeding, the faeces were collected from the bottom of the aquarium at certain time intervals and all the complete seeds were washed-out and counted. A plastic screen separated the fish from the bottom of the aquaria, so that the excreted seeds were not eaten twice. The retrieved seeds were set to germinate in petri dishes (5.2 cm) on a double layer of Whatman No. 1 filter paper, moistened with 2.0 ml of tap water. Germination was tested during a period of 30 days in a growth chamber under constant temperature (25° C), photoperiod (14h) and irradiance (100 µE m^{-2} s^{-1} PAR). Germination of the seeds which passed through the digestive system of the fish was compared with the germination of untreated control seeds. Germination of *Ruppia* was investigated in batches of 200 seeds whereas that of *Najas* seeds was in multiples of 50 or 100.

Three species of fish were selected: *Oreochromis* sp. (tilapia or St. Peter's fish); *Ctenopharyngodon idella* (the grass-carp) and *Cyprinus carpio* (the common carp). Tilapia is a native fish of Israel. It is of an unpure population which is genetically close to *Oreochromis aurea.* The two carps are introduced species which, at present are found in fish ponds and water reservoirs all over the country (Leventer 1984, 1987). The common carp is known to be a "bottom grazer" with an efficient food chewing and grinding system. The grass-carp was introduced for aquatic weed control (Terrell 1975; Leventer 1984).

Experiments were conducted either with hungry fish (4 days without food) or with well-fed ones. Ten fish were used per aquarium and the figures denote averages for all ten specimens.

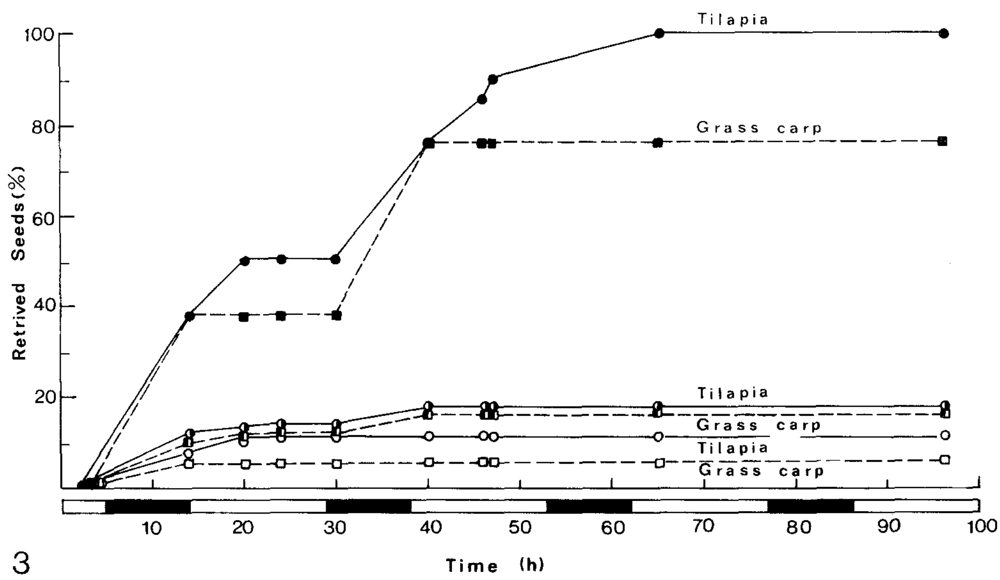
Seed-coat hardiness was estimated from its resistance to pressure. Individual seeds were placed below a set of weights that were gradually increased. The exact weight which caused the seed coat to crack was recorded.

The structure of the seed-coats of the three types of seeds of *Najas marina* was analyzed by SEM (Jeolco, JSM-35) after fracturing of frozen seeds and coating them with gold.

Results

A mixed batch of seeds of *Najas marina* and uniform hard seeds of *Ruppia maritima* were used in the first set of experiments. The three species of fish differed in their effects on the consumed seeds (Fig. 1). The common carp (*Cyprinus carpio*) had ground the seeds almost completely, excreting but small fragments of the seed coats. Only very few seeds passed through the digestive tract of the common carp without being destroyed.

The grass carp (*Ctenopharyngodon idella*) was less destructive and excreted some 30–40% of the seeds complete.



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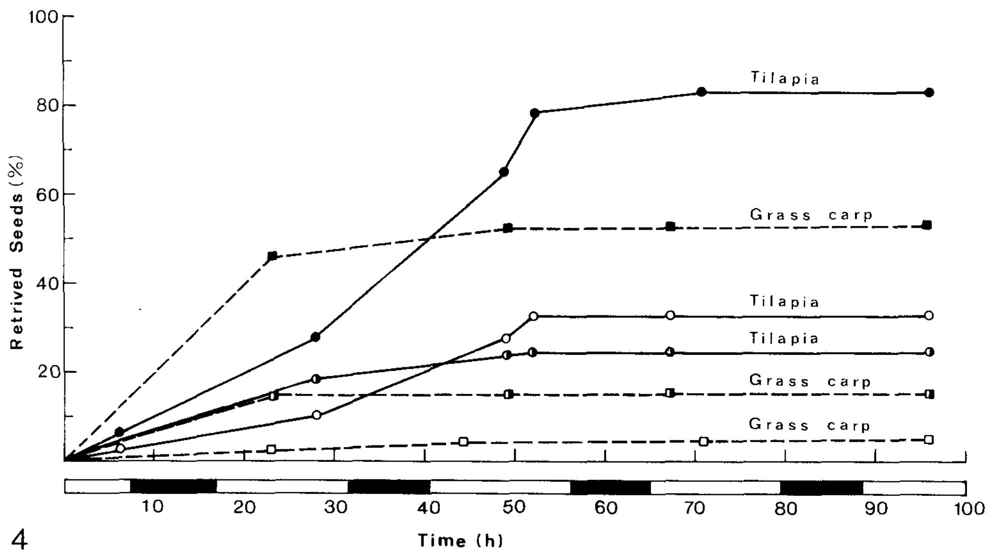

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Figs. 3 and 4. Time course of seed retrieval of *Najas marina. Full symbols* – hard seeds; *semi-full symbols* – light-colored seeds; *empty symbols* – soft seeds. Day and night hours are marked on the abscissa. Fifty seeds of each type were ingested by each of the two fish species. **3** – Hungry fish; **4** – Satisfied fish

The smallest amount of damage was inflicted on the seeds by the tilapias (*Oreochromis* sp.), with some 60% of the eaten seeds being excreted without damage (Fig. 1).

In the second set of experiments the seeds of *Najas* were divided into three distinct types: "soft seeds", "light seeds" and "hard seeds" (Fig. 2). The seeds of each group were fed to the different fish separately. Feeding was not repeated with the common carp. Results are presented in Figs. 3 and 4. A clear difference in retrieval of seeds of the various types was observed. While very few of the soft and the light seeds were retrieved, less than 20% of the ingested seeds (Fig. 3), the hard coated ones were retrieved in large numbers (80% retrieval from grass carp faeces and almost full retrieval from the faeces of the tilapias).

Excretion of the ingested seeds was not uniform during the day. Most of the excretions occurred during the night, especially during the first two days after feeding and by satisfied fish. Daytime excretions of seeds were rare. The seeds were excreted gradually; some seeds were retained inside the intestines of the fish for periods which varied between 30 and 65 h, depending on the species.

Another factor that controls the effects of fish on the seeds is the feeding status (Figs. 3 and 4). Hungry fish consumed more of the soft and light seeds of *Najas*, but were unable to digest most of the hard ones. A similar phenomenon was observed with seeds of *Ruppia* where satisfied fish consumed a smaller number of seeds than hungry ones (Fig. 5).

Germination of seeds of *Najas* and of *Ruppia* was highly variable (Table 1). While germination of untreated seeds was scattered and did not exceed 0.5%, the seeds that passed through the digestive tract of the fish germinated in higher percentages: Germination of *Ruppia* reached 12.2% after passing the tilapias and 5.7% after the grass-carp. Germination percentages of the retrieved hard seeds of *Najas* were even higher: 16% after passing tilapias and 10.5% after the grass-carp. It is interesting to note that none of the soft seeds that were retrieved intact from the faces have germinated. Passage of such seeds through the digestive system of the fish killed them even without an apparent mechanical damage. Some 40% of the light-colored seeds contained undeveloped embryos.

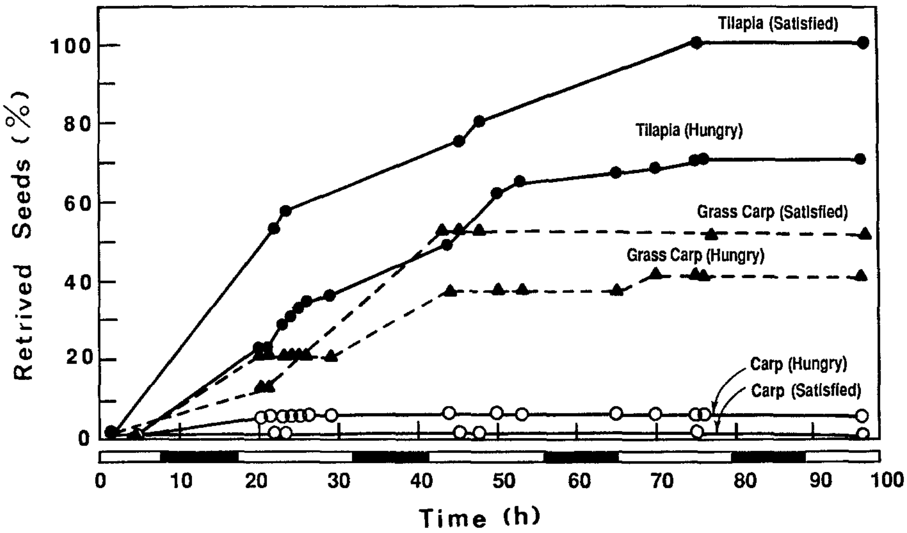

Fig. 5. Time course of *Ruppia maritima* seed retrieval from hungry and satisfied fish. Two hundred seeds were ingested by each of the three fish species. Day and night hours are marked on the abscissa

Table 1. Germination percentage of the 3 types of seeds of *Najas marina* (50 seeds of each type) and seeds of *Ruppia maritima* (200 seeds) as affected by the duration of stay in the digestion tract of hungry tilapias

Time inside the digestion tract	Germination (%)			
	Najas marina			*Ruppia maritima*
	Hard seeds	Light seeds	Soft seeds	Black seeds
0 h (control)	0	0	0	0.5
20 h	24	0	0	6.4
47 h	5	0	0	18.5
65 h	20	0	0	11.6
Mean germination	16	0	0	12.2

Table 2. Variations in size of the three types of *Najas marina* seeds and in their mechanical strength (Mean ± SD; 20 replications)

Seed type	Length (mm)	Width (mm)	Pressure that caused cracking (gr)
Hard seeds	2.97 ± 0.36	1.77 ± 0.27	3245 ± 245
Light seeds	3.01 ± 0.27	1.78 ± 0.25	1110 ± 171
Soft seeds	2.67 ± 0.28	1.22 ± 0.19	420 ± 120

Great differences were found, in size and in the seed-coat resistance to pressure, among the different types of seeds of *Najas marina* (Table 2). Seed-coats of the hard seeds were cracked only by pressures which were 8 times higher than those that cracked the soft seeds. The light seeds were in an intermediate position.

The differences in structure between the seed-coats of the three types of seeds of *Najas marina* can be seen in Fig. 6. The coats of the hard seeds are comprised of 8–10 layers of stone-cells, whereas those of the soft or light seeds are 4–5 cell layers thick only. The walls of the hard-seed-cells are composed of several layers and were much thicker than those of the other seed coats.

Discussion

The relationships between fish and plants are of a complex nature. Fish do not swallow water-plant-seeds as a gesture of altruism. Fish feed on such seeds and therefore, one would expect their full digestion. Nevertheless, if all such seeds would have been consumed, the plants might have faced extinction and the fish famine. Thus, a compromise has been reached between the demand of fish for food and the demand of plants for long distance dispersal and for improvement of germination (see also Owen 1980; Belsky 1986; Agami and Waisel 1986). However, not all fish behave accordingly. The common carp is a total destroyer of seeds. Also, tilapias, and especially hungry ones, consumed seeds (cf. Bowen 1982) but mainly of soft or light ones. Grass-carps are efficient digestors of all types of seeds either when hungry or when satisfied. Apparently, many fish species consume some seeds but transport and excrete the rest of them. Thus, it is tempting to assume that an ecological meaning hides beyond the production of the two types of seeds by *Najas:* (1) the soft and easily digested "bait type" seeds that attract the hungry animals and (2) the hard and often also undigestable "dispersal type" of seeds, on which plants may depend for their reproduction and distribution (see also Faegri and Pijl 1971, regarding stamens and insects). Such seeds are so structured as to endure the passage through the animals' digestive tracts, with minimal damage to the embryo but with an impact on the seed-coat that improves consequent germination (cf. Howe 1986; Howe and Smallwood 1982; Estrada and Fleming 1986).

The apparent differences in mechanical strength of the seed-coats of the three types of seeds, are reflected by their structure. The seed-coats of all three groups of seeds are constructed of stone cells, elongated along the long axis of the seeds. The thicker the seed-coat is and the thicker the cell-walls of the stone cells are, the more durable are the seeds.

The three species of fish which we have investigated cannot be considered as major dispersal agents for aquatic plants. Firstly, because one of them, the common carp, fully digests the ingested seeds. Secondly, the two other species do not migrate over large distances. However, within the limited area that is covered by such fish, the dispersal

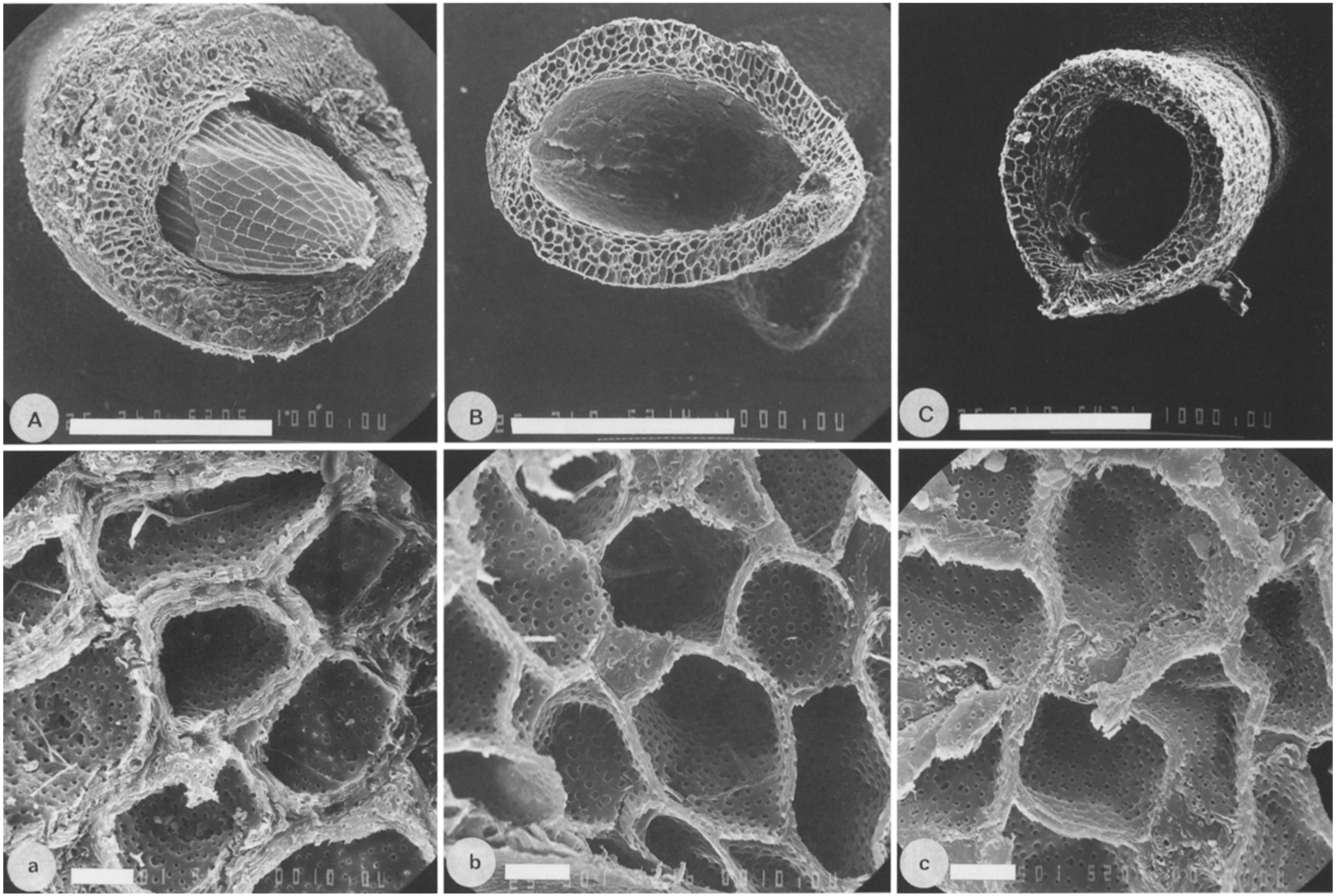

Fig. 6 A–C, a–c. Seed-coat structure of the three types of seeds of *Najas marina*. **A, a** – hard seeds, **B, b** – light colored seeds; **C, c** – soft seeds. SEM image. Bar **A, B, C** = 1 mm. Bar **a, b, c** = 20 μm

of seeds into new and unoccupied sites might be of significant value. Moreover, once a year, during the breeding season, such fish migrate to distant hatching places, and thereby may expand the distribution area of the plants. Another contribution of the fish to the plant-fish interaction is the improvement of seed germination. Germination of seeds of many aquatic plants is extremely slow. Improvement of germination by a factor of 5–10, by the passage of seeds through the digestive tracts of animals, would thus be of significance. It is interesting to note that the improvement of germination did not depend upon the duration of stay that the seeds stayed inside the fish intestines; the major effect was exerted by the mechanical impact of ingestion rather than by the long exposure to the low pH of the fishes' stomachs.

Dispersal of seeds inside the digestion tracts of fish is not only a function of digestibility; it depends very much upon the time of consumption: seeds ingested towards the night stand a greater chance to be excreted locally, whereas seeds ingested in the morning may travel long distances. The variability in digestibility of seeds by various fish has its practical value (cf. Robson 1977; Leventer 1984, 1987). The common carps digest almost all the ingested seeds but have little effect on the vegetative organs of *Najas* or of *Ruppia*. Under such conditions, the common carp destroys the basis for its future supply of food – excluding habitats where plant reproduction depends upon vegetative organs (Agami et al. 1986). On the other hand, the grass-carps and tilapias consume large quantities of the vegetative organs of both macrophytes, but digest only some of the seeds while improving the germination of the others. Thus, one may regard the behavior of these fish as being of a "farming" type, i.e., fish whose future supply of food is ensured by their own feeding habits. Such a combination of fish with different feeding habits might be of practical value for biological control of aquatic weeds.

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