Response to abiotic stress in *Pomacea canaliculata* with emphasis on cold tolerance

Takashi Wada¹ and Keiichiro Matsukura²

¹Suya 1635-107, Koshi, Kumamoto 861-1102, Japan. Email: wadat520@gmail.com
²NARO Kyushu Okinawa Agricultural Research Center, Koshi, Kumamoto 861-1192, Japan. Email: mtkr@affrc.go.jp

Abstract

*Pomacea* apple snails have invaded temperate East Asia as well as tropical Southeast Asia. Cold winter temperatures undoubtedly limit the distribution of *P. canaliculata* in temperate regions. Snowfall might also prevent hibernation. However, *P. canaliculata* enhances its cold tolerance before the onset of winter. Winter snails were approximately nine times more cold-tolerant than summer snails, based on the time to 50% mortality at 0°C. Gradually decreasing temperatures in autumn and increasingly dry conditions are the environmental cues for enhancement. Physiologically, cold-tolerant snails in winter accumulate glycerol in their bodies, while glycogen levels decrease. Cold tolerance is linked with desiccation tolerance: cold tolerant snails survive longer after desiccation exposure than cold intolerant snails. After winter, warm temperatures together with irrigation prior to rice planting remove the cold tolerance of overwintered snails in temperate paddy fields. When paddy fields are drained before harvest, the snails bury themselves into the soil or move under rice straw. This behaviour is effective for avoiding cold and desiccation. It is apparent that *P. canaliculata* cannot colonize temperate paddy fields without physiological enhancement of cold tolerance together with behavioural avoidance. *Pomacea canaliculata* is more tolerant of cold and desiccation than is the other important invasive apple snail, *P. maculata*, such that *P. canaliculata* may be more adapted to ephemeral Asian paddy fields.

**Additional keywords:** Ampullariidae, desiccation, hibernation, invasive species, *Pomacea maculata*, Mollusca, salinity, temperature
Introduction

*Pomacea canaliculata* and *P. maculata* (junior synonym *P. insularum*) are widely distributed invasive species originating in South America. The former is listed as among 100 of the world’s worst invasive species (Lowe *et al*., 2000). Abiotic environmental stresses (e.g. climate) are major factors determining success of their colonization of new regions. For tropical and subtropical species, cold weather in winter undoubtedly limits colonization and range expansion in temperate regions. Although the natural distribution of *P. canaliculata* extends further south (to cooler regions) than any other South American *Pomacea* species (Seuffert *et al*., 2010), expansion of its range in east Asia has been restricted by cold winter weather (Ito, 2002; Yoshida *et al*., 2009, 2013). Here, we focus primarily on cold tolerance in *P. canaliculata*, the mechanism of which has been well studied in Japan (Wada & Matsukura, 2007, 2011; Matsukura & Wada, 2007; Matsukura *et al*., 2008, 2009a, b), but also touch on studies dealing with other abiotic stresses.

**Seasonal adaptation to cold in Pomacea canaliculata**

*Pomacea canaliculata* was introduced to Japan in the early 1980s (Mochida, 1991; Wada, 2004). Snails in paddy fields in temperate Japan increase their cold tolerance before the onset of winter (Fig. 1; Wada & Matsukura, 2007; Matsukura & Wada, 2007).

![Fig. 1. Seasonal fluctuation of cold tolerance (survival when exposed to low temperatures) in P. canaliculata juveniles collected from Japanese paddy fields (submerged and drained) at different times of year.](image-url)
This increase is not great, compared to other examples of cold adaptation in many insects and some molluscs (Lee, 1991; Ansart & Vernon, 2003), but is nonetheless important. Assessing survival at 0 °C for five days is a good approach to detect changes in cold tolerance. No snails collected from submerged paddy fields in summer survived this treatment, while most snails overwintering in drained fields survived. Snails overwintering in waterways also enhance cold tolerance in winter. Winter snails were approximately nine times more cold-tolerant than summer snails, based on the time (survival days) to 50 % mortality at 0 °C (Wada & Matsukura, 2007). Generally, juveniles of intermediate size (10-20 mm) are more cold tolerant than smaller juveniles (< 10 mm) or adults (Syobu et al., 2001; Wada & Matsukura, 2007).

Not only snails from temperate Japan but also snails from the tropical Philippines that had never experienced cold temperatures through many generations over the three decades since their introduction responded to cold acclimation by enhancing cold tolerance (Wada & Matsukura, 2011). Since snails collected from northern Argentina showed the same response (Yoshida et al., 2014), adaptation to cold temperatures in P. canaliculata is a trait originating in snails in South America rather than a trait evolved after introduction into temperate Asia (Seuffert et al., 2010; Wada & Matsukura, 2011).

Mechanism of cold tolerance

Gradually decreasing temperatures in autumn is the main environmental cue to enhance cold tolerance (Matsukura & Wada, 2007). Dry conditions (depriving the snails of water) enhances cold tolerance to some extent even at 25 °C. Photoperiod shows no clear effect on cold tolerance.

After winter, temperature and water availability lead to decreasing cold tolerance of overwintered snails (Matsukura et al., 2009b). Under aquatic conditions, cold tolerance broke down within four days at 25 °C and eight days at 20 °C. However, snails held at 15 °C retained their cold tolerance more than 64 days. Snails kept under dry conditions maintained their cold tolerance for at least 64 days even at 25 °C. These results indicate that warm temperatures together with irrigation before rice planting lead to loss of cold tolerance of overwintered snails in these temperate paddy fields.

Physiologically, cold-tolerant snails in winter accumulate glycerol and possibly glucose in their bodies, while glycogen concentration decreases (Matsukura et al., 2008). Among low molecular-weight compounds besides glycerol and glucose, increases in glutamine and carnosine and a decrease in phenylalanine were observed. It is generally
considered that in insects and other organisms concentrations of low molecular-weight compounds such as polyols and sugars increase with advancement of cold tolerance in winter (Lee, 1991; Storey & Storey, 1997). In molluscs, such increases have been reported in only a few instances: the intertidal snail *Melampus bidentatus*, the intertidal bivalve *Mytilus edulis* (Loomis, 1985; Loomis *et al*., 1988) and the land snail *Helix pomatia* (Nowakowska *et al*., 2006). In general, low molecular-weight compounds decrease supercooling points, acting as cryoprotectants (Lee, 1991; Storey & Storey, 1997). Other possible functions related to enhancement of cold hardiness are to prevent protein denaturation by hydrogen-bonding, to prevent membrane damage by inhibition of lipid phase transition (Michaud *et al*., 2008; Sjursen & Somme, 2000) and to reduce cuticular water loss by binding water (Williams & Lee, 2008). But no difference in supercooling points was observed in *P. canaliculata* between snails with and without cold tolerance (Matsukura *et al*., 2009a), possibly because the glycerol concentration in cold tolerant *P. canaliculata* is much lower than in many insects (Lee, 1991; Matsukura *et al*., 2008). *Pomacea canaliculata* cannot tolerate freezing (Matsukura *et al*., 2009a). But this fact seems ecologically unimportant because death occurred experimentally and in fields at much milder temperatures (0-10 °C) than that at which the snails freeze (ca. -7 °C) (Wada & Matsukura, 2007; Matsukura & Wada, 2007; Matsukura *et al*., 2009a). Even in other organisms, mechanisms of lethal injury by chilling (mild low temperatures) in the absence of freezing have been rarely understood as compared to freezing tolerance. Thus, the actual function of glycerol in *P. canaliculata* is unknown.

At the histological level, the mantle is the most susceptible among various organs examined to sustain injury from cold temperatures (Matsukura *et al*., 2009a). In cold-tolerant snails, decrease of glycogen and increase of glucose in the kidney, increase of glucose in the mantle and increase of glycerol in foot muscle and digestive glands were prominent.

**Hibernation in new Asian habitats and the significance of enhancing cold tolerance**

*Pomacea canaliculata* has invaded both temperate East Asia and tropical Southeast Asia. Its main habitat in these regions is ephemeral paddy fields, where submerged and dry conditions cycle with rice cultivation and intervening fallow periods. In more temperate regions the snails hibernate during winter, adopting at least two tactics. One is the physiological enhancement of cold tolerance described above. The other
is behavioural avoidance of cold and desiccating conditions. When paddy fields are drained before harvest, snails bury into the soil or after harvest move under rice straw. Temperatures under straw are much milder than at the exposed soil surface. In an experiment carried out in Kyushu (southern Japan), temperatures under straw were almost always above 0 °C, when soil surface temperatures frequently went down below -5 °C (Fig. 2; Wada & Matsukura, 2007). However, many snails fail to bury and these snails, even with physiological cold tolerance, are killed during winter (Kiyota & Sogawa, 1996). Adult snails often cannot bury entirely because of their large size. Due to this imperfect behaviour together with size dependent cold tolerance and the higher possibility of shell breakage caused by tillage, there are very few adult survivors after winter in temperate paddy fields. Therefore, the great majority of snails that appear at the first irrigation of the fields are juveniles of intermediate size (10-22 mm shell height) (Wada et al., 2004). Mortalities during winter are usually very high (85 %, Watanabe et al., 2000; 92 %, Wada & Matsukura, 2007; 91-99 %, Yoshida et al., 2009), indicating that *P. canaliculata* could not colonize these temperate paddy fields without physiological cold enhancement combined with behavioural avoidance.

![Graph showing daily maximum and minimum temperatures](image)

**Fig. 2.** Daily maximum and minimum temperatures measured at three sites in paddy fields (on the soil surface, under rice straw and 1.2 m above soil surface) during the 2005-2006 winter in Kyushu, Japan.
Influence of temperature on growth and activity

In *P. canaliculata* growth and activity including feeding and crawling are entirely suppressed below 10 °C (Kaneshima *et al.*, 1987; Seuffert *et al.*, 2010). Juvenile developmental time (1/juvenile period) increases linearly between 15 and 30 °C, while growth is reduced at 35 °C compared to 30 °C (Kaneshima *et al.*, 1987). Activity also declines above 30 °C and the snails fall into heat coma at 36.2 °C (Seuffert *et al.*, 2010). The lower temperature thresholds and cumulative heat requirement for embryonic development ranged between 16 and 18 °C and between 89 and 134 day-degrees, respectively (Seuffert *et al.*, 2012). At 0 °C 50 % mortality occurred in 1.3 days for summer snails and 11.9 days for winter snails (Wada & Matsukura, 2007). At -3 °C about 50 % of snails died in 2 days. The supercooling point of the snails was between -6.6 and -7.1 °C (Matsukura *et al.*, 2009a). Nonetheless, incubation for long periods at temperatures of 0-10 °C is fatal (Matsukura & Wada, 2008, Wada unpublished data) and thus such temperatures are ecologically much more important than the value of the supercooling point. Furthermore, the cumulative number of day-degrees below 10 °C (CNDDb10) during winter is sometimes used to assess overwintering success (Ozawa & Akino, 1988; Yoshida *et al.*, 2009). Heat shock proteins, which may work to reduce temperature stress, have been studied by Zheng *et al.* (2012) and Giraud-Billoud *et al.* (2013). Although the literature is limited, lethal temperatures in other ampullariiids were summarized by Cowie (2002).

Some recent implications for hibernation and distributions

In temperate Asia cold weather limits *P. canaliculata* distribution (Ito, 2002; Yoshida *et al.*, 2009, 2013). However, mere mean winter temperatures or CNDDb10 do not always explain actual distributions. In central-southern Japan *P. canaliculata* inhabits paddy fields in the areas facing the Seto Inland Sea (Sanyo and southern Kinki Districts; Fig. 3) where the mean temperatures in the coldest months generally ranges from 2 to 6 °C (Fig. 4). The areas with the same winter mean temperatures are widely distributed in the regions facing the Sea of Japan (Sanin and Hokuriku Districts; Fig. 4). However, *P. canaliculata* does not occur in these areas except at a location (Togo town) having hot-springs (Fig. 3; Wada, 2015). In addition, several examples of the disappearance of *P. canaliculata* following colonization have been recorded in Sanin and Hokuriku. There
Fig. 3. County-based distribution of *P. canaliculata* in selected prefectures in central-southern Japan. The counties coloured dark are those where *P. canaliculata* was found in paddy fields. Areas A and B correspond to the areas on the map in Fig. 4. The distribution map was drawn using data from surveys conducted by Prefectural Plant Protection Offices mainly in 2011 (modified from Wada, 2015).

Fig. 4. Mean temperatures in the coldest month (January) in central-southern Japan: A, Sanin and Sanyo Districts; B, Hokuriku District. The map was drawn using the software Mesh 2012 (Japan Meteorological Business Support Center, Chiyoda-Ku, Tokyo) (modified from Wada, 2015).
is a marked difference in climate during winter between the two areas facing the Sea of Japan and the Seto Inland Sea: Sanin and Hokuriku have a snowy, rainy and cloudy climate, while sunny days with large diurnal temperature ranges often continue in Sanyo and southern Kinki into winter. Snow cover keeps the soil surface at approximately 0 ℃ for long periods (Fukui, 1977). Continuous low temperatures close to zero are critical for the snails. All hibernating snails died experimentally at 0 ℃ for 20 days (Wada & Matsukura, 2007). In addition, snails under conditions of small diurnal temperature fluctuations died after far fewer CNDDb10 than snails under highly fluctuating temperature conditions (Wada, unpublished data). Thus, a climate with frequent snowfall and temperatures with small diurnal range are suspected to prevent snails from overwintering in Sanin and Hokuriku even though the winter mean temperatures seems to be appropriate for overwintering.

Occurrence of hybrids between *P. canaliculata* and *P. maculata* was demonstrated based on analysis of the nuclear EF1α DNA marker by Matsukura *et al.* (2013). *Pomacea canaliculata* is more cold tolerant than *P. maculata* (Yoshida *et al.*, 2013; Matsukura *et al.*, 2016), and therefore *P. canaliculata* occurs in both tropical and temperate Southeast and East Asia, whereas *Pomacea maculata* is confined to tropical and subtropical regions. Hybrids, however, inhabit both tropical and temperate regions but do not reach the northern parts of the *P. canaliculata* distribution. Hybrids produced experimentally through back-crossing of F1 to *P. canaliculata*, exhibited intermediate cold tolerance between the two species. This phenomenon helps to explain the current distribution of the three types of *Pomacea* snails in East and Southeast Asia. It is possible that *P. maculata* genes expanded northwards through hybridization (Matsukura *et al.*, 2016).

**Response to other abiotic stresses**

*Desiccation*

Many ampullariids bury themselves and enter aestivation when their habitats dry up (Cowie, 2002). Desiccation tolerance therefore seems important for the snails’ ability to survive such conditions. Field collected *P. canaliculata* survived 11 months in dry soil in a laboratory experiment (Yusa *et al.*, 2006a). In dry conditions but with occasional moistening (a shower two or three times a month) they survived up to 29 months. Juveniles of intermediate size (ca. 15 mm) were more tolerant than smaller juveniles (ca. 8 mm) and adults (ca. 30 mm). These results are relevant to the time it will take for
total snail mortality to be achieved following adoption of an upland, non-irrigated crop, as a control tactic (Wada et al., 2004).

Cold-acclimated laboratory-reared juveniles (ca. 11 mm) exhibited ca. 20 % survival 17 weeks after the start of a desiccation treatment at 25 °C and 25-55 % relative humidity (Wada & Matsukura, 2011). Moreover, linkage of cold tolerance and desiccation tolerance is found in the progenies of both temperate and tropical snails: cold-tolerant snails survive longer after desiccation exposure than cold intolerant snails. There are often similarities in mechanisms underlying adaptation to cold temperatures and desiccation; notably, accumulation of metabolites including polyols and sugars, adjustments of water content and modification of habitat preferences (Ring & Danks, 1994; Michaud et al., 2008). Pomacea canaliculata was more tolerant to desiccation than P. maculata, suggesting that P. canaliculata is more adapted to ephemeral Asian paddy fields (Yoshida et al., 2014).

Survival of Pomacea paludosa in relation to seasonal desiccation in Florida wetlands has been investigated and relevant aspects of water management have been discussed by Darby et al. (2003, 2008).

**Salinity**

Although P. canaliculata does not occur in salty and very alkaline environments and inhabits shallow quite turbid sites with a low Na⁺/(K⁺+Mg²⁺) ratio in its native Argentina (Martín et al., 2001), the snails seems to be somewhat tolerant of salinity. Kijima et al. (1988) investigated survival of small juveniles in a range of salinities. Although tolerance was different among strains, some mortality occurred in 0.2 % salt water and the majority were dead in 0.4 % (but mortality was very low for one strain). Cowie (2002) suggested P. canaliculata was sufficiently tolerant of sea water to survive long enough to spread to nearby river systems. But in northern Peninsular Malaysia, sea water irrigation of paddy fields was successfully adopted to eradicate newly invaded snails (Yahaya et al., 2006).

**Conclusions**

Pomacea canaliculata accumulates glycerol in its body and enhances cold tolerance before the onset of winter. When paddy fields are drained, the snails bury themselves in the soil or move under rice straw, where they hibernate in a much buffered environment. The capability of physiologically increasing cold tolerance together with behavioural
avoidance of cold and desiccation are major factors enabling this species to expand its range into temperate East Asia. *Pomacea canaliculata* is more tolerant to cold and desiccation than *P. maculata*, which may indicate that *P. canaliculata* is better adapted to ephemeral Asian paddy fields, the major habitat of these invasive apple snails. A major constraint on spread and abundance in tropical regions seems to be drought, but there are still few studies dealing with desiccation stress. Biotic factors are also important even in the newly invaded habitats. Numerous predators of *P. canaliculata* are known in Japan and some may effectively regulate local populations (Yusa et al., 2006b; Yamanishi et al., 2012). Biotic factors as well as abiotic factors should be considered when success or failure in colonization by invasive organisms is discussed.

References


Matsukura, K., Okuda, M., Cazzaniga, N.J. & Wada, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading
East and Southeast Asia. *Biological Invasions* 15: 2039-2048.


Yusa, Y., Sugiura, N. & Wada, T. 2006b. Predatory potential of freshwater animals on an invasive agricultural pest, the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) in southern Japan. *Biological Invasions* 8: 137-147.