

Regular paper

Distribution and settlement of *Ruditapes philippinarum* in the Suo-Nada Sea, Japan

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Running headline: Manila clam larval distribution and settlement

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(Abstract should be on page 2)

Abstract

Understanding the causes of spatiotemporal variations in the scale of larval settlement is important for population dynamics studies in bivalves. This study investigated the seasonal abundance of the asari or Manila clam (*Ruditapes philippinarum*) larvae over a 4-year period (2004–2007) and their settlers over 3 years (2005–2007) in the Suo-Nada Sea, Japan. Seasonal differences in larval transport were examined by numerical simulation using 3D ocean-modelling. During the 2004–2007 spawning seasons, larval numbers peaked two or three times in June/July, August/September, and October/November. Settler occurrence was uncoupled with larval occurrence; settler density was >10 times higher in October/November than in other months. Numerical simulation suggested that the extent of larval transport differed seasonally; larval transport via loss from the Suo-Nada Sea was estimated to be 20% in June/July, whereas it was almost 0% in November. However, this could not explain the seasonal difference (>10-fold) in settler density. In addition, the average density of larger larvae (>180 μm) during 2004–2007 was higher in June/July than in October/November, as a result of spawning and larval loss (via transport and mortality), suggesting that larval supply alone could not explain the seasonal differences in settler density. These results suggest that the seasonal differences in settler density were affected more by variation in mortality during the settlement and/or early post-settlement stages, which may depend on environmental conditions at the settling site, rather than by larval supply.

Keywords: clam, larval supply, larval transport, settlement

Introduction

For many marine bivalves including the asari or Manila clam *Ruditapes philippinarum* (Adams & Reeve 1850), spatio-temporal differences in larval supply constitute a potential cause of variations in the scale of larval settlement (Young *et al.* 1998; Shanks & Brink 2005). Understanding the causes of variations in larval supply and settlement, along with post-settlement processes, is important for studies on the population dynamics of bivalves (Hunt & Scheibling 1997; Pineda, Hare & Sponaugle 2007).

R. philippinarum was originally distributed in the temperate to subarctic regions along the east coast of the Pacific Ocean, but is currently found in many other areas worldwide because of its introduction for aquaculture (Goulletquer 1997). Population studies on *R. philippinarum* have attracted attention because of its commercial and ecological importance, especially after a population decline in Japan (Miyawaki & Sekiguchi 1999; Ishii *et al.* 2001; Toba *et al.* 2007; Tamaki *et al.* 2008; Tezuka *et al.* 2012). Spawning and larval occurrence of the asari clam has been observed during spring to autumn at water temperatures above 14 °C (Miyawaki & Sekiguchi 1999; Ishii *et al.* 2001; Matsumura *et al.* 2001; Drummond, Mulcahy & Culloty 2006). Dense larval settlement has often been recorded in autumn in temperate regions of Japan (Miyawaki & Sekiguchi 1999; Ishii *et al.* 2001; Toba *et al.* 2007). However, the causes of seasonal variation in settler density and the decoupling between larval occurrence and settlement are as yet unclear.

The Suo-Nada Sea was renowned as one of the major *R. philippinarum* fishery ground in Japan until the population drastically declined in the late 1980s (see Tezuka *et al.* 2012, 2014). Surveys conducted in the 1970s and early 1980s, before the population decline, showed that larval abundance peaked in spring and autumn, and that settlers were observed mainly in autumn (Inoue 1980; Fujimoto *et al.* 1985). No larval surveys have been carried

out after the population decline; however, seasonal observations of settlement on the Nakatsu tidal flats in the Suo-Nada Sea have been reported recently by Tezuka *et al.* (2012), and settlers have been observed in autumn.

To ascertain the causes of seasonal differences in settler density observed on the Nakatsu tidal flats during 2005–2007 by Tezuka *et al.* (2012), we investigated the abundance and distribution of *R. philippinarum* larvae between April and November over the period 2004–2007. In addition, seasonal differences in larval transport were examined by numerical simulation. Data for settler numbers were obtained from the Tezuka *et al.* (2012) study and reanalyzed to determine the periods of larval settlement using shell-length distribution. The study will increase our understanding of the causes of seasonal differences in settler density and larval numbers after the clam population decline in the Suo-Nada Sea.

Materials and Methods

Sampling of larvae and settlers

Sampling of *R. philippinarum* larvae was conducted in the Suo-Nada Sea between April and November over the period 2004–2007 (Fig. 1). Larvae were collected by pumping 200 L of seawater from a 5-m depth and screening onto a 50- μ m mesh net. The sampling depth was changed to an intermediate level in cases where the sampling stations were shallower than 10 m. In the periods 19–22 June 2007, 20–23 August 2007, 15–19 October 2007, and 16–19 November 2007, multilayer sampling from 2-, 5- and 10-m depths was conducted to ascertain the vertical distribution of clam larvae. Samples were frozen until needed for identification, enumeration and shell length measurement. After thawing, larvae were identified using fluorescent antibodies and counted under a fluorescence microscope (Matsumura *et al.* 2001;

Kasuya, Hamaguchi & Furukawa 2004). Shell length was measured using an eyepiece to the nearest 10 μm , and up to 100 individuals were measured per sample.

R. philippinarum settlers were collected at two stations on the Nakatsu tidal flats during the period 2005–2007 (Fig. 1; see Tezuka *et al.* 2012) by taking three, 1-cm deep surface-sediment samples with a core sampler (40 mm in diameter). Samples were preserved by freezing until settlers were counted and shell lengths measured to the nearest 0.1 mm.

Numerical simulation of larval transport

A 3D numerical ocean model was used for simulation of *R. philippinarum* larval transport in the Suo-Nada Sea. Particle-tracking experiments were conducted using the same flow-field reported by Chang *et al.* (2009). A total of 1000 modelled larval particles were set in each of nine areas along the Suo-Nada coast (Fig. 2) and tracked by the Euler–Lagrange method for 14 days (100 particles per 10-model grids per area). The simulation was conducted over two seasonal periods: June/July (from June 30 to July 17) and November (from November 7 to November 27).

The position of the particle $X_{n+1}(x^{n+1}, y^{n+1})$ at time $n+1$, which was $X_n(x^n, y^n)$ at time n , can be calculated by the following equation:

$$X_{n+1} = X_n + V\Delta t + (\partial V/\partial t + V\nabla V)\Delta t^2/2 + R$$

where V denotes the horizontal velocity vector of residual flow; Δt , the time step; ∇ , the horizontal gradient. R is the dispersion due to turbulence and is given by the following equation:

$$R = \gamma(2\Delta t D_h)^{1/2}$$

where γ is the normal random number whose average is zero and whose standard deviation is 1.0. D_h is the horizontal dispersion coefficient that depends on Smagorinsky diffusivity (Mellor 2004), which varies with horizontal shear stress.

Vertical migration of larval particles was hypothesized as larvae were located at a 3-m depth from day 0 to day 11 but, then moved to 1 m above the ocean floor between day 12 and day 14. This assumption for the vertical migration of larval particles is based on observational studies on the vertical distribution of larvae, including this study (see Results, Fig. 6), which showed that smaller larvae (D- and umbo-shaped) were found at ~3 m, whereas larger larvae (settling larvae) found in the bottom layer (Suzuki *et al.* 2002; Ishii, Sekiguchi & Jinnai 2005; Kuroda 2005; Toba *et al.* 2012; Bidegain *et al.* 2013). The larval stage was assumed to last 2 weeks (14 days) in this study, although it can vary with temperature and food availability (Helm & Bourne 2004).

The larval retention rates, i.e., the ratio of larval particles remaining within the Suo-Nada Sea, after the 14-day simulation were calculated as follows:

$$\text{Retention rate after 14 days} = 100 \times P_a / P_0$$

where P_a is the number of particles remaining within the Suo-Nada Sea after the 14-day simulation, and P_0 is the number of particles within the Suo-Nada Sea on Day 0. The boundary of the Suo-Nada Sea was set on a line through two points (131.7°E, 33.7°N) and (132.0°E, 34.0°N) (see Fig. 1), and particles located to the western side of the boundary line were treated as inside the Suo-Nada Sea. Retention rates were calculated for each of the nine areas where particles were released for two seasonal periods.

Results

Seasonal dynamics of larval distribution and settlement

Distribution of *R. philippinarum* larvae from April to November 2004–2007 is shown in Fig. 3. Seasonal changes in planktonic larval abundance are shown in Fig. 4. *R. philippinarum* larvae were observed sporadically from April to November, with peak numbers being

recorded two or three times in a year, i.e. June/July, August/September and October/November (Figs. 3 and 4). Larvae were distributed more widely in the Suo-Nada Sea during June/July (e.g. 13–16 June 2005, 19–2 June 2006 and 19–22 June 2007) than in October/November (e.g. 20–23 November 2005, 30 October–2 November 2006 and 15–19 October 2007) (Fig. 3). The maximum recorded planktonic larval density was c. 2000 individuals m^{-3} , observed in November 2006 and in June 2007 (Fig. 4).

The size-frequency distribution of *R. philippinarum* settlers on the Nakatsu tidal flats between May–December 2005–2007 is shown in Fig. 5. New settlers (< 0.5 mm in shell length) were found almost exclusively in October/November for the 3-year period (Fig. 5). The density of new settlers (< 0.5 mm in shell length) differed by more than 10-fold between summer and autumn: 200–1000 individuals m^{-2} in June–August, and 4000–13,000 individuals m^{-2} in October/November.

Vertical distribution of *R. philippinarum* larvae, inferred from the multilayer sampling trials, are shown in Fig. 6. During 19–22 June 2007, smaller larvae (120–180 μm in shell length) were more abundant at the 2- and 5-m depths than at 10-m, whereas larger larvae (>180 μm) were more abundant at 10 m. During the other time-periods, larvae were less abundant than during 19–22 June 2007, being found almost exclusively at 2 m.

Numerical simulation of larval transport

Results of the numerical simulation of larval particle-tracking are shown in Fig. 7. In the June/July period, released larval particles were carried extensively by two major currents that developed in the Suo-Nada Sea; a circular current in the central region and a northward current along the western coast. In the November simulation, the currents had weakened and the larval particles were retained within the Suo-Nada Sea. The retention rates for particles within the Suo-Nada Sea after the 14-day simulations for June/July and November are shown

in Table 1. In the June/July period, retention rates of larval particles varied by area where the particles were released, with rates ranging from 32.2% (Area 4) to 100% (Areas 5 and 6). On average, 79.5% of the particles were retained within the Suo-nada Sea, while the rest were lost by being transported from the Suo-Nada Sea in the June/July period. For the November period, retention rates were higher than in the June/July period at >99%.

The densities of planktonic larvae of different sizes are compared between June/July and October/November for combined data (2004–2007) (Fig. 8). The average larval density of all combined size classes was 79.2 individuals m^{-3} in June/July and 36.3 individuals m^{-3} in October/November, i.e., approximately twice as higher in the former. Of these, larger larvae (>180 μm) constituted 8.0% in June/July (6.3 individuals m^{-3}) and 10.2% in October/November (3.7 individuals m^{-3}).

Discussion

This study reported the seasonal dynamics of *R. philippinarum* larval distribution in the Suo-Nada Sea and the number of settlers on the Nakatsu tidal flats. Larval occurrence and settlement events were not necessarily coupled and, although larvae were recorded from April to November, settlement occurred almost exclusively in October/November. Numerical simulation suggested that larval loss via current transport was higher in June/July than in November. However, the actual density of larger larvae (>180 μm) was higher in June/July than in October/November, suggesting that the supply of larvae was not the cause of seasonal differences in settler density.

The seasonal occurrence pattern of larvae and settlers observed in this study was similar to that reported in previous studies within the Suo-Nada Sea and other regions. Inoue (1980) reported that planktonic larval density peaked in May and October/November in 1976

on the northern coast of the Suo-Nada Sea (maximum larval density was 14,000 individuals m^{-3} in October 1976), and that new settlers were found in abundance (26,700 individuals m^{-2} in November 1976) in October/November, but very few, new settlers were recorded in other months. Fujimoto *et al.* (1985) observed that larval density peaked in April, June/July, September and November during the period 1983–1984 on the southwest coast of the Suo-Nada Sea, with a maximum density of 4550 individuals m^{-3} in November 1983 and 6140 individuals m^{-3} in April 1984 (larval density was calculated as the mean from eight sampling stations). An increase in new settlers was observed in October/November 1983 (maximum of 350,000 individuals m^{-2} in November 1983) and in November/December 1984 (max, 20,000 individuals m^{-2}). Although the maximum larval density observed in the current study was lower than those recorded in the 1970s and 1980s, the seasonal pattern was consistent among these studies. The difference in maximum larval density could be a result of differences in the spawning biomass between the 2000s and the 1970–1980s; clam production in the Suo-Nada Sea was 14,800 metric tonnes in 1976, 19,500–31,700 metric tonnes in 1983/1984, and 94–780 metric tonnes during 2005–2007 (Ministry of Agriculture, Forestry and Fisheries, Japan, 2012). Dense larval settlement in autumn has often been observed in other temperate regions of Japan (Miyawaki & Sekiguchi 1999; Ishii *et al.* 2001; Toba *et al.* 2007).

The results of numerical simulation suggest that larval retention in the Suo-Nada Sea should be higher, i.e., lower larval loss rates, in October/November than June/July, owing to seasonal differences in the strength of two major currents in the Suo-Nada Sea. As stratification developed in June/July, a circular current developed in the central region and a northern current developed along the southwest coast of the Suo-Nada Sea. However, as stratification declined in October/November, the currents weakened. The simulation suggested that larval loss via transport from the Suo-Nada Sea was 20% in June/July and 0% in October/November. However, this disparity could not explain the >10-fold difference in

settler density between October/November and June/August. Moreover, on average during the period 2004–2007, the observed density of >180 μm larvae was higher in June/July than in October/November. This suggests that, even if the larval loss via transport may be higher in June/July, larval supply, as a result of spawning and larval loss via transport and mortality, was not the limiting factor in June/July under the prevailing conditions.

Factors influencing settler density, other than larval supply, need to be considered, and although not all factors were investigated in this study, several hypotheses may be proposed. First, mortality during the critical period from settling to early post-settlement affects settler density (Hunt & Scheibling 1997), and this mortality may be lower in October/November than in other months. Predation and/or other factors, such as high temperature in summer, may cause higher mortality in settling larvae or early settlers during months other than October/November. Second, food availability for larvae may differ seasonally, resulting in differences in mortality during settlement (Laing 1995; Tezuka *et al.* 2009). Third, the rate of larval settlement may vary due to seasonal differences in physical conditions at settling sites, e.g., variation in sediment grain size (Tezuka *et al.* 2013). If a suitable substrate is not found, larvae could delay settlement (Coon, Fitt & Bonar 1990) and, as a result, become more prone to predation by benthic filter-feeders while drifting near the bottom (Pineda *et al.* 2010). This study concentrated solely on larval settlement on the tidal flats and other possible sites, e.g., subtidal areas, were not investigated. As such, settlement failure on the tidal flats in seasons other than October/November needs to be investigated further.

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339

Table 1 Retention rate (%) of particles within the Suo-Nada Sea after a 14-day simulation. Retention rates were calculated for each of the nine area where particles were released over two seasonal periods, June/July and November.

Area	1	2	3	4	5	6	7	8	9	Ave.
June/July	85.4	93.7	40.4	32.2	100	100	75.6	89.9	98.7	79.5
Nov	99.7	100	100	100	100	100	100	100	99.4	99.9

(Each Table should be on a separate sheet.)

Figure legends

Fig. 1 (a)-(b), Map of the Suo-Nada Sea, Japan. Dashed line in (b) is the boundary used to calculate the larval retention rate (see text). (c)-(d), Nakatsu tidal flats where larval settlement was recorded.

Fig. 2 Modelled larval particles were set in nine areas along the Suo-Nada Sea coast for numerical simulation of *Ruditapes philippinarum* larval transport. A total of 1000 modeled larval particles were set on 10-model grids in each area. The model grids in each area were indicated as “+” symbols with identical color.

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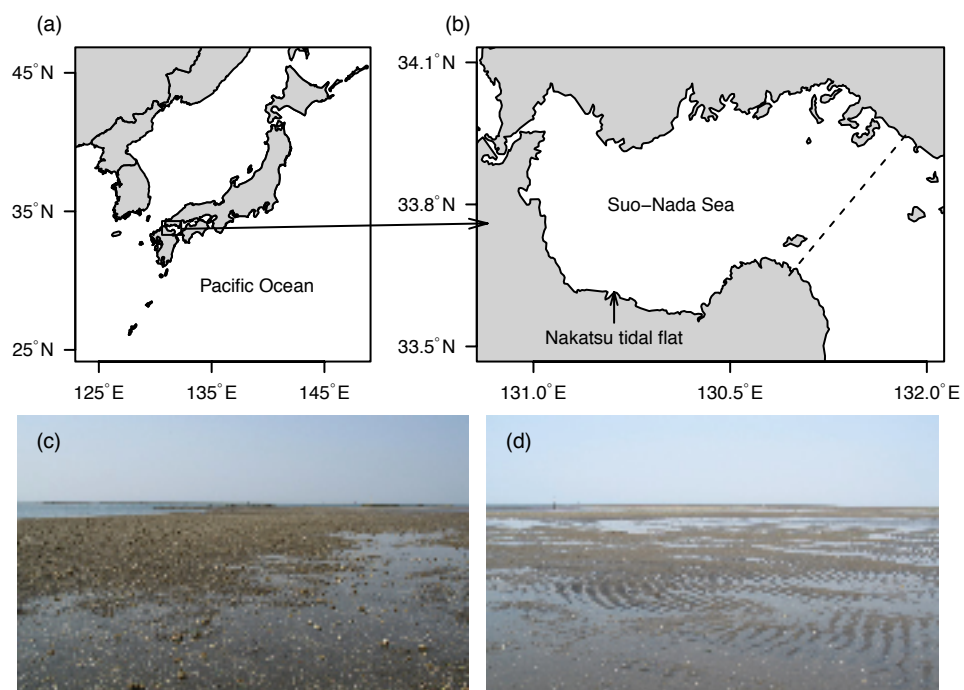


Fig 1

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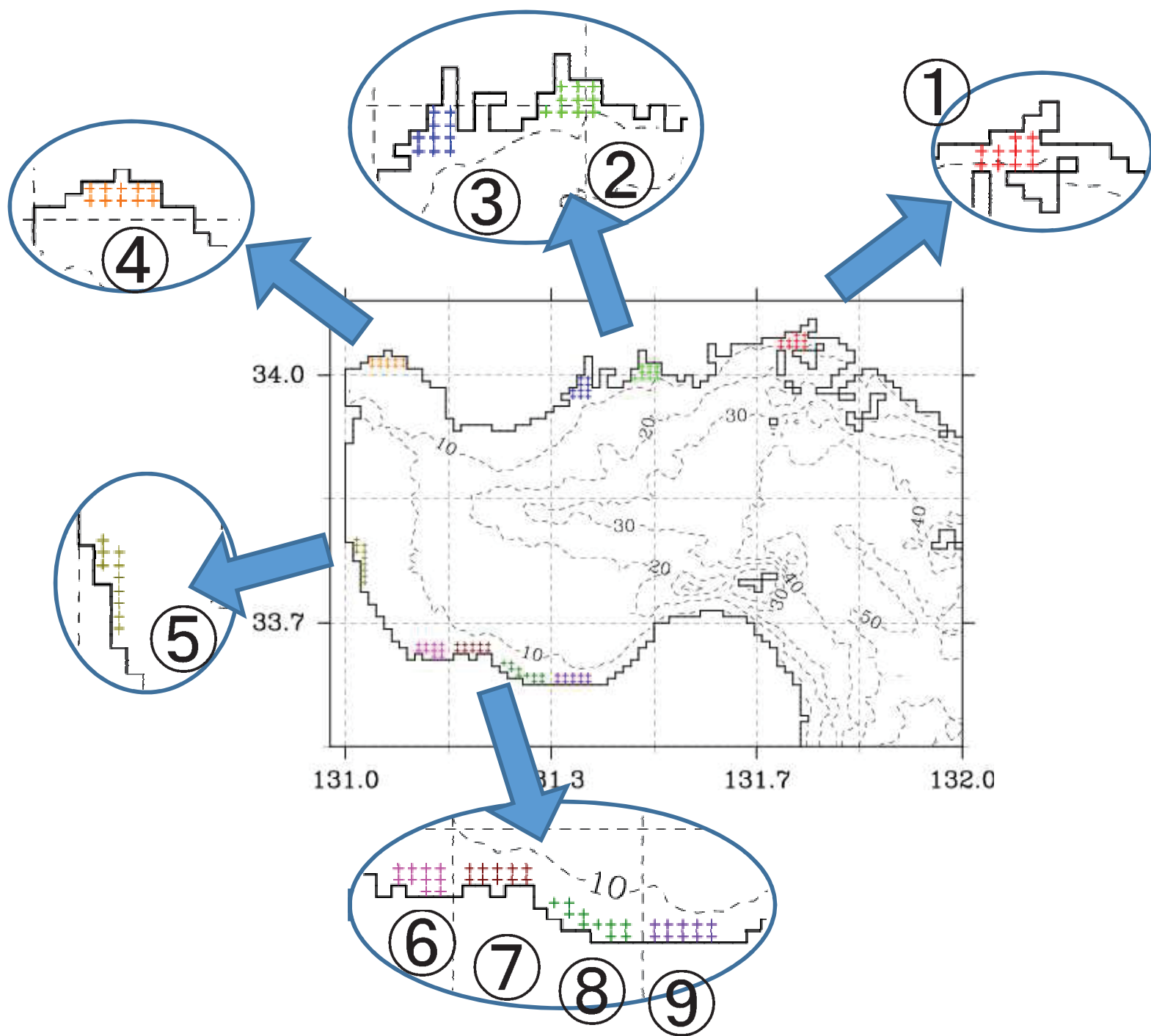


Fig 2

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