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240 **Dead heat: Copepod carcass occurrence along the Japanese coasts and implications for**
241 **a warming ocean**

242

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253

254 *Abstract:*

255 Understanding global warming effects on marine zooplankton is key to proper management

256 of marine resources and fisheries. This is particularly urgent for Japan where the coastal

257 water temperature has been increasing faster than the global average over the past decade.

258 Conventional sampling and monitoring programmes, by ignoring the *in situ* vital status of the

259 zooplankton, produce incomplete information about the state of the ecosystem. We showed

260 that marine copepod carcasses were ubiquitous along a latitudinal gradient of 34–39 °N of the

261 Japanese coasts. On average, 4.4–18.1% of the individuals of the main copepod genera

262 (*Acartia*, *Paracalanus*, *Oithona* and *Pseudocalanus*) were carcasses, equivalent to 19–250 µg

263 C m⁻³. Higher fractions of dead copepods tended to occur at higher water temperatures,

264 implicating temperature-dependent non-predation mortality. Carcass occurrence may

265 represent a loss of copepod production for the traditional predation-based food chain. On
266 average 49.5% of the carcass carbon would be remineralised in the water column via bacteria
267 respiration, with the remainder potentially exported to the seafloor. Continuous warming in
268 the Japanese coasts is expected to accelerate non-predation copepod mortality, with unknown
269 consequences for the local marine food web.

270

271 *Keywords:* Marine copepods, vital status, non-predation mortality, global warming, carcass
272 decomposition

273

274

275 **Introduction**

276 Given the trophic importance of zooplankton (e.g., copepods) for the marine food web
277 and fisheries, studies of zooplankton species compositions and abundances have long been a
278 key component of oceanographic research, and programmes of long-term sampling and
279 monitoring of marine zooplankton have been established around the world (Mackas *et al.*,
280 2012). The conventional sampling methods used in these programmes, however, do not
281 provide information on the *in situ* vital status of the zooplankton. It is a common practice for
282 researchers to indiscriminately preserve and count all zooplankton in field samples as live
283 individuals, and subsequently apply the data to derive biological rates (e.g., grazing,
284 excretion, reproduction, etc.) for the zooplankton populations. It is, however, incorrect to
285 assume all zooplankton in the ocean are alive. While conventional research tends to
286 emphasise predation as a primary force in shaping zooplankton life histories and population
287 structures (Verity and Smetacek, 1996), a comprehensive meta-analysis has shown that non-
288 predation factors account for up to one-third of pelagic adult copepod mortality (Hirst and
289 Kiørboe, 2002), which may leave carcasses behind. The available literature data show that
290 12–60% (averages) of the zooplankton populations in the ocean may be dead (Tang *et al.*,

291 2014). Failure to determine zooplankton vital status will therefore introduce enormous errors
292 into zooplankton population rates and related ocean models (Elliott and Tang, 2011a).

293 The recent advance of the Neutral Red (NR) staining method has provided researchers
294 with a quick and simple way to separate live and dead marine zooplankton (especially
295 copepods) in field samples (Elliott and Tang, 2009; Zetsche and Meysman, 2012). The
296 method has since been applied in various places, such as the Chesapeake Bay (Elliott and
297 Tang, 2011b), the Mediterranean Sea (Besiktepe *et al.*, 2015), the English Channel (Maud *et*
298 *al.*, 2018), the Humboldt Current (Yanez *et al.*, 2018) and the Sargasso Sea (Tang *et al.*,
299 submitted). Knowledge of the live/dead composition of the zooplankton populations may
300 reveal alternative pathways for the zooplankton biomass. For example, bacteria may colonize
301 and decompose zooplankton carcasses, thereby diverting zooplankton biomass to the
302 microbial food web (Tang *et al.*, 2006; Bickel and Tang, 2010). Conversely, carcasses may
303 sink and contribute to carbon flux to the benthos (Ivory *et al.*, 2014, Tang *et al.*, submitted).
304 Some recent studies have shown that zooplankton carcasses can make up a major fraction (up
305 to 91%) of the carbon sinking flux, especially outside of the phytoplankton bloom periods
306 (Frangoulis *et al.*, 2011; Sampei *et al.*, 2009, 2012).

307 The Japanese coastline covers a large latitudinal range, where the strong climate
308 gradient plus the specific local environmental conditions may lead to large spatial and
309 temporal variations in the coastal zooplankton communities. Ongoing warming in Japanese
310 coastal waters (Japan Meteorological Agency; <https://www.data.jma.go.jp>) is expected to
311 adversely affect marine zooplankton. It is therefore increasingly important for researchers to
312 determine the zooplankton's vital status in field surveys to obtain a better understanding of
313 the changing marine environment, and the consequences for fisheries and ecosystem
314 functioning. Ocean warming will increase zooplankton's metabolic rate leading to shorter
315 life-spans. Additionally, each zooplankton species can be characterized by its temperature

316 tolerance threshold, above which will be fatal (González, 1974). Temperature-dependent
317 mortality in zooplankton (e.g. copepods) has been studied, but usually in the context of large
318 (and sudden) increase in temperature (e.g. Jiang *et al.*, 2009; Choi *et al.*, 2012), whereas
319 increase in zooplankton mortality along a natural temperature gradient has rarely been
320 investigated. Nevertheless, two recent papers have shown an increase in copepod mortality in
321 relation to temporal (decadal and seasonal) increase in water temperature: The total mortality
322 rate of *Calanus helgolandicus* at station L4 in the English Channel increased with rising
323 temperature over the past decade (Maud *et al.*, 2018). Likewise, both predation and non-
324 predation mortality rates of *Acartia tonsa* increased with the seasonal increase in water
325 temperatures in the Chesapeake Bay (Elliott and Tang, 2011a). Based on those results, we
326 may predict that the percentages of dead zooplankton, indicative of non-predation mortality,
327 would increase along a spatial gradient of water temperature. Our study offered the first
328 opportunity to test this prediction in Japanese coastal waters among the different zooplankton
329 populations.

330 In this study, we sampled the zooplankton at five coastal bays around the main island of
331 Japan, covering a latitudinal gradient of 34.1 to 39.3 °N. We used the NR staining method to
332 distinguish between live and dead copepods, and examined their relationships with the
333 environmental conditions. To gain insights into the fate of the copepod carcasses, we also
334 conducted experiment to measure microbial decomposition rates of the carcasses, based on
335 which we estimated carcass carbon turnover rates.

336

337 **Materials and Methods**

338 Field sampling

339 Sampling was done between May and July, 2013, at different coastal locations in Japan
340 (Table 1). Except for Tokyo Bay, each location had multiple sampling points (Figure 1).
341 Hamanako estuary and Otsuchi Bay were each sampled in two different months.

342 At each sampling point, zooplankton were collected by duplicate vertical tows, from ca.
343 1 m off the bottom to the surface, with a standard ring net (200 μm mesh; 0.48 m diameter
344 mouth opening). The cod-end content of the first tow was preserved in formalin for species
345 identification and enumeration. The cod-end content of the second tow was treated on board
346 with NR stain following the protocol of Elliott and Tang (2009), then concentrated on 200
347 μm meshes and kept on ice until return to the laboratory.

348 In the laboratory, the NR-treated samples were examined under the microscope.
349 Zooplankton that were stained red (i.e., individuals that were alive at the time of collection)
350 and the ones that were unstained (i.e., carcasses) were preserved separately in formalin.
351 Because of the high copepod species diversity in Japanese coastal waters and that some
352 species occurred only sporadically, to facilitate data analysis and comparisons, we grouped
353 the species into major genera (*Acartia*, *Paracalanus*, *Oithona*; plus *Pseudocalanus* in Otsuchi
354 Bay) when measuring the live/dead ratios. Other minor taxa were analysed for their total
355 abundances but not live/dead compositions. *In situ* carcass abundances were calculated from
356 total abundances (first net tows) and the live/dead ratios (second net tows). Random
357 subsamples of the major copepod genera were measured for prosome lengths, from which we
358 estimated the individual carbon weights according to Uye (1982).

359 Water temperature, salinity, dissolved oxygen and chlorophyll *a* were measured
360 vertically at 0.5 m intervals from surface to 1 m above the bottom by submersible sensors at
361 the same time as zooplankton collection; results were presented as mean values for the water
362 column.

363 Carcass decomposition experiment

364 Experiment was conducted using surface seawater and *Oithona* spp. collected from
365 Sagami Bay. Surface seawater was passed through a 5- μm sieve to remove large organisms
366 and detritus but keep the ambient microbial community; this filtered seawater (FSW_{5 μ}) was
367 used for the incubations.

368 In the laboratory, live individuals of *Oithona* spp. were sorted. The copepod dry weight
369 was measured as 2.45 $\mu\text{g ind}^{-1}$ and the corresponding carbon content was estimated as 1.10
370 $\mu\text{g C ind}^{-1}$ (Uye, 1982). Fresh carcasses were produced just before the experiments by
371 dipping the live copepods in 1 M HCl for a few seconds; the carcasses were then rinsed
372 copiously with FSW_{5 μ} and transferred to glass incubation vials containing FSW_{5 μ} (4
373 carcasses per vial; each vial ca. 4.5 mL). Four vials with carcasses were used as treatments;
374 four with only FSW_{5 μ} were used as controls. The vials were incubated in a 26°C water bath
375 (same as *in situ* water temperature at the time of experiments) and were individually mixed
376 by a magnetic stirrer (Unisense). Carcass decomposition by ambient microbes was measured
377 as oxygen consumption using an optic-fibre microsensor (Unisense). Before the experiments,
378 the microsensor was calibrated with 100% and 0% oxygen-saturated FSW_{0.2 μ} . At time 0, the
379 optic fibre was inserted into the vials through a special opening in the stoppers to measure the
380 dissolved oxygen. Each measurement lasted ca. 2 min. The process was repeated at 30, 60
381 and 120 min. O₂ consumption rates were calculated between consecutive time intervals (i.e.,
382 0-30 min, 30-60 min, 60-120 min); 'excess' microbial respiration was then calculated as the
383 difference between the treatment vials and the control vials. Bacterial carbon consumption
384 rates were estimated by assuming 1:1 O₂-to-C molar ratio and 45% bacterial growth
385 efficiency (Del Giorgio and Cole, 1998); carcass carbon turnover rates were then calculated
386 as carcass carbon weight divided by microbial carbon consumption rates, corrected for *in situ*
387 temperature with a Q₁₀ value of 1.88 (Lomas *et al.*, 2002).

388

389 **Results**

390 Environmental conditions

391 Mean temperature for the water column at each sampling location ranged from 9.5 to
392 26.1 °C during our sampling period (Figure 2) and was noticeably higher at Hamanako
393 estuary where the water body was more sheltered than the other locations. The water was
394 considerably cooler in the northern-most location (Otsuchi Bay). Slight increases in water
395 temperature were detected between June and July in Hamanako estuary, and between May
396 and July in Otsuchi Bay. Water salinity ranged from 31.3 to 33.8, and was slightly lower in
397 Seto Inland Sea, Hamanoko estuary and Tokyo Bay owing to the influence of small
398 freshwater runoff at these locations. The water columns were in general well oxygenated,
399 with the mean dissolved oxygen concentration at $\geq 6 \text{ mg L}^{-1}$, although water masses with
400 lower oxygen concentrations ($4.3\text{-}5.7 \text{ mg L}^{-1}$) were observed in Hamanako estuary and Tokyo
401 Bay, where chlorophyll *a* concentrations were also considerably higher than in the other
402 locations, indicative of the eutrophic status of the waters.

403 Combining the environmental data from all locations, both salinity ($r^2 = 0.17$) and
404 D.O. ($r^2 = 0.87$) correlated negatively with temperature ($p < 0.05$), whereas chlorophyll *a*
405 concentration correlated positively with temperature ($r^2 = 0.35$, $p < 0.05$).

406

407 Copepod community compositions

408 Besides copepods, several zooplankton taxa were present but only sporadically and
409 were not included in the analysis; these include invertebrate larvae (gastropod, bivalve,
410 polychaete, barnacle), amphipod, decapod, chaetognath and appendicularian. The one non-
411 copepod group that occurred regularly in substantial amounts was Cladocera (*Penilia*, *Evadne*
412 and *Podon* spp.). However, our preliminary tests showed that live cladocerans did not take up
413 NR stain very well, and they were also easily damaged by the handling, giving false

414 negatives (incorrectly identified as dead). Therefore, cladocerans were also excluded from
415 further analysis.

416 The copepod communities were dominated by calanoid copepods, followed by
417 cyclopoid and harpacticoid copepods. To illustrate the geographical differences in copepod
418 community compositions, we calculated the percentages (based on numerical abundances) of
419 the prominent species/taxa that accounted for at least 5% of the total abundance in each of the
420 Order of Calanoida, Cyclopoida and Harpacticoida at each location (Table 2). In the case of
421 Hamanako estuary and Otsuchi Bay, where we sampled more than once, we also present the
422 results for the different months to illustrate the temporal changes in the copepod community
423 compositions.

424 Among the calanoid copepods, *Acartia omorii* was the main species in Hamanako
425 estuary (July) and Tokyo Bay, accounting for nearly half of the calanoid copepods. It was
426 also quite common in Seto Inland Sea (12%) and Sagami Bay (24%), but was rare to absent
427 in Otsuchi Bay. *Paracalanus parvus* was noticeably abundant in Seto Inland Sea (40%),
428 whereas Otsuchi Bay was characterized by the prominent presence of *Pseudocalanus*
429 *newmani* (5%) and *Pseudocalanus* copepodites (22–47%). Small percentages of
430 *Clausocalanus pergens* and *Clausocalanus* copepodites were present in Otsuchi Bay in May,
431 and they became quite abundant in July (10–15%). Calanoid copepodites were generally
432 abundant at all locations. In Hamanako estuary, the percentages of *Acartia* and *Paracalanus*
433 young copepodites decreased between June and July, accompanied by an increase in the
434 percentages of *A. omorii* and *P. parvus* adults in the July samples.

435 Among the cyclopoid copepods, *Oithona davisae* was particularly numerous in
436 Hamanako estuary (55% in July) and Tokyo Bay (38%). In Otsuchi Bay, the dominant
437 *Oithona* species were *O. similis* and *O. atlantica* (9–25%). *Corycaeus affinis* was also a
438 noticeable part of the cyclopoid community, especially in Seto Inland Sea (32%) and Otsuchi

439 Bay (24% in July). Copepodites of *Oithona* and *Corycaeus* species were highly abundant.
440 Harpacticoid copepods were less abundant and were dominated by just a few species, most
441 notably *Euterpina acutifrons*, *Microsetella norvegica*, *M. rosea* and *Tigriopus japonicus*.

442 Copepod live/dead compositions

443 For the analysis of live/dead compositions, we focused on the main genera of *Acartia*,
444 *Paracalanus* and *Oithona*, which were present in substantial amounts across all locations,
445 plus *Pseudocalanus* in Otsuchi Bay (Table 3). There were considerable variations in the
446 genera's numerical abundances (live + dead) between locations as well as within locations.
447 Overall, *Acartia* was numerically the most common, and it was particularly abundant in
448 Sagami Bay (4200 ind m⁻³) and Otsuchi Bay (1400 ind m⁻³). *Paracalanus* and *Oithona* were
449 also ubiquitous: the former was the most abundant in Seto Inland Sea (1300 ind m⁻³), whereas
450 the latter was particularly abundant in Otsuchi Bay (1300 ind m⁻³). *Pseudocalanus* was also
451 highly abundant in Otsuchi Bay (2300 ind m⁻³).

452 The fractions of dead copepods were quite variable between locations as well as within
453 locations (Table 4). The highest average % dead was found in Hamanako estuary for all three
454 main genera (*Acartia*, *Paracalanus*, *Oithona*). In the case of *Oithona*, as much as 53%
455 (average) of the copepods were carcasses in Hamanako estuary. The global average % dead
456 across all sampling locations ranged from 4.4 to 18.1%.

457 Despite the relatively small % dead in *Acartia* and *Pseudocalanus*, the large individual
458 body sizes still resulted in relatively large total carcass carbon concentrations, which were on
459 average an order of magnitude higher than that of *Paracalanus* and *Oithona* (Table 5).

460 For each genus, carcass carbon concentration varied considerably within a location
461 (Figure 3), mainly a result of patchy distribution of carcasses rather than differences in

462 individual body sizes in the samples. All three genera suffered considerably higher % dead in
463 Hamanako estuary; consequently, the amount of carcass carbon was relatively high, despite
464 the modest copepod abundances there compared to the other locations.

465 *Paracalanus* was present in Otsuchi Bay, but its carcasses were rare to undetectable. In
466 comparison, *Paracalanus* carcass carbon was quite prominent in Hamanako estuary. *Oithona*
467 abundance was particularly high in Otsuchi Bay, and so was the corresponding amount of
468 *Oithona* carcass carbon. The amount of *Pseudocalanus* carcass carbon, which was present
469 primarily in Otsuchi Bay, was quite comparable between May and July.

470

471 Copepod carcass decomposition

472 Microbial decomposition of the copepod carcasses led to lower dissolved oxygen levels
473 in the treatment vials relative to the control vials (Figure 4). The microbial respiration rate on
474 copepod carcasses averaged across the three time-intervals was 7.59 nmol O₂ h⁻¹. Assuming
475 1:1 O₂-to-C molar ratio and 45% bacterial growth efficiency (Del Giorgio and Cole, 1998),
476 the average bacterial carbon consumption rate on carcasses was 0.20 μg C h⁻¹. Applying this
477 value to the estimated carcass carbon weight (1.10 μg C ind⁻¹), the carcass carbon turnover
478 rate due to microbial decomposition was 18.4% h⁻¹.

479

480 **Discussions**

481 Temperature is a fundamental driver of biological processes, but its effects on
482 zooplankton non-predatory mortality has rarely been studied *in situ* (Elliott and Tang, 2011a;
483 Maud *et al.*, 2018). This study was the first attempt to investigate copepod carcass
484 occurrence—indicative of non-predation mortality, across the large latitudinal (hence
485 temperature) gradient along the Japanese coasts.

486 Diverse coastal zooplankton communities were observed in this study. *Acartia omorii*
487 was common among the calanoid copepods. This species was historically confused with *A.*
488 *clausi*, but was later identified as a separate species, and has been found widely distributed in
489 Japanese coastal waters (Ueda, 1986). In Otsuchi Bay, *A. hudsonica* replaced *A. omorii* as the
490 main coastal *Acartia* species, especially in May—this is consistent with an earlier report
491 (Nishibe *et al.*, 2016). *Paracalanus parvus* is another common coastal species found in this
492 study. The precise taxonomy of this species is still in debate, with ‘major’ and ‘minor’
493 morphological forms described by earlier investigator (e.g., Hirota, 1964), and more recent
494 genetic analysis has suggested a *P. parvus* species complex in Japanese water that comprises
495 of multiple species (Hidaka *et al.*, 2016). This species *sensu lato* was abundant in Seto Inland
496 Sea in our study, similar to previous reports (Yamazi, 1952; Hirota, 1969). It was relatively
497 rare in Otsuchi Bay in May, but became a noticeable component of the calanoid community
498 in July, as it has been observed by others (Nishibe *et al.*, 2016). Among the cyclopoid
499 species, the relatively high abundances of *Oithona similis* and *Corycaeus affinis* in Seto
500 Inland Sea in the month of June resemble what was observed decades earlier (Hirota, 1964).
501 The common occurrence of *Oithona* species, especially *O. davisae* and *O. similis*, in our
502 sampling areas also agrees with other reports (e.g., Yamazi, 1952; Itoh *et al.*, 2011; Nishibe
503 *et al.*, 2016).

504 Among the three main genera (*Acartia*, *Paracalanus* and *Oithona*), only % dead
505 *Acartia* showed a significant, positive correlation with chlorophyll *a* concentrations (Figure
506 5). The very high chlorophyll *a* concentrations were found in warm and highly eutrophic
507 Hamanako estuary, where potentially harmful dinoflagellates have been observed, such as
508 *Gymnodinium* spp. (Hanai *et al.*, 1992), *Alexandrium* spp. (Narita *et al.*, 1999) and *Ceratium*
509 spp. (our own observations). While some harmful algal blooms are known to cause
510 physiological, behavioural and/or reproductive impairments, it is less clear whether they

511 directly cause mortality in copepods (Turner and Tester, 1997; Turner, 2014). It is more
512 likely that the observed correlation was an indirect relationship with temperature. Likewise,
513 although fluctuations in salinity and D.O. concentration are known to affect *Acartia* survival
514 in extreme cases (e.g., Calliari *et al.*, 2008; Elliott *et al.*, 2013), the rather narrow range of
515 values of salinity and D.O. concentration would suggest that the negative correlations were
516 likely driven by temperature (Figure 5).

517 For all three copepod genera, the values of % dead were quite variable, but the higher
518 values tended to occur at temperatures over 21°C (Figure 5). Higher temperatures could
519 increase the copepods' metabolic rate leading to shorter life-spans (i.e., higher natural death,
520 which is a form of non-predation mortality). Metabolic rate should vary with temperature in
521 an exponential fashion, as usually described by Q_{10} . In the case of *Acartia*, however, the data
522 were better described by a power function. If we were to fit an exponential function to the
523 observations, the equivalent Q_{10} would be 6.3, which seems unrealistically high. Conversely,
524 when the environmental temperature exceeds a certain threshold of tolerance, mortality could
525 spike resulting in a large increase in % dead (Jiang *et al.*, 2009; Choi *et al.*, 2012). Our
526 observations suggest that, in addition to a potentially higher natural death promoted by
527 warmer temperatures, the three main copepod genera along the Japanese coasts may have an
528 upper thermal limit of ca. 21°C, above which non-predation mortality increased sharply
529 resulting in much higher fractions of dead copepods in the water column. In the Chesapeake
530 Bay, a high proportion of dead copepods occurred during a summer heat spell (Tang *et al.*,
531 2006), and non-predatory copepod mortality rates also increased with water temperature
532 (Elliott and Tang, 2011a). Hence, our findings and the earlier findings together suggest that
533 temperature is a strong driver (predictor) of non-predation mortality and carcass occurrence
534 among coastal copepods, both in time and in space.

535 Research on global warming effects on marine zooplankton is often focused on changes
536 in the organisms' abundance, phenology and distribution (e.g., Roemmich and McGowan,
537 1995; Edwards and Richardson, 2004; Mackas *et al.*, 2007; Richardson, 2008), but how
538 zooplankton mortality rates and patterns may change in a warming ocean has received
539 relatively little attention. According to the latest report by the Japan Meteorological Agency
540 (<https://www.data.jma.go.jp>), the annual average sea-surface temperature around Japan has
541 risen by +1.11°C between 1900 and 2017, which is more than the average warming for the
542 global ocean and the N. Pacific. Our results showed that the mean summer water
543 temperatures in areas south of Otsuchi Bay were already higher than or close to the
544 hypothetical upper thermal limit of 21°C. If the warming trend continues, we may expect to
545 see a surge in non-predation mortality among the local copepod populations. However, to
546 fully understand the consequences on the marine food web and fishery, we would also need
547 to consider potential changes in copepod species composition and primary production.
548 Compounding the problem, the Japanese coasts are under other anthropogenic pressures
549 (Ministry of the Environment; <https://www.env.go.jp>) which, when acting synergistically
550 with warming, may further exacerbate non-predation mortality (e.g., Sokolova and Lannig,
551 2008; Elliott *et al.*, 2013). To better manage and protect Japanese coastal ecosystems, it is
552 therefore imperative for researchers to study the response of zooplankton mortality rates and
553 patterns in the changing ocean environment, and for monitoring programmes to include
554 measurements of zooplankton vital status.

555 One ecological fate of copepod carcasses is decomposition by ambient microbes (Tang
556 *et al.*, 2006; Bickel and Tang, 2010). The amounts of copepod carcass carbon estimated in
557 this study were much lower than the typical seston carbon concentrations in coastal waters
558 (on the order of 100 mg m⁻³; Tang *et al.*, 2000). However, the C:N molar ratio of copepod
559 biomass is ca. 4.5 compared to ca. 7 for seston, which makes copepod carcasses a favourable,

560 protein-rich substrate source for bacteria. Our experiments showed that the copepod carcasses
561 were quickly decomposed by bacteria, resulting in elevated respiration rate in the ambient
562 water. The relative amount of carcass carbon being remineralised within the water column vs.
563 exported to the seafloor is determined by microbial respiration rate and the residence time of
564 the carcass in the water column. We used a Q_{10} value of 1.88 to estimate the carcass
565 decomposition rates at the *in situ* temperatures of the different sampling locations (Lomas *et*
566 *al.*, 2002). Using the summed carcass carbon concentrations of the main genera at the
567 different locations (Table 5), and assuming a carcass sinking rate of 4.3 m h^{-1} (for
568 copepodites of similar sizes; Elliott *et al.*, 2010), we calculated the % carcass carbon lost to
569 microbial respiration in the water column, and the corresponding amounts of carcass carbon
570 available for export to the seafloor (Table 6).

571 Both Tokyo Bay and Otsuchi Bay are relatively deep sampling locations (26 and 32.1
572 m, respectively). However, the two places are on the opposite ends of the spectrum in terms
573 of copepod carcass carbon export potential: The warmer temperature in Tokyo Bay (hence
574 faster decomposition) plus the low carcass abundance resulted in the lowest amount of
575 carcass carbon for export ($0.15 \mu\text{g C m}^{-2}$). In contrast, microbial respiration was at a much
576 lower rate in the colder water of Otsuchi Bay, allowing the largest amount of carcass carbon
577 available for export to the seafloor (7.46 mg C m^{-2}).

578 Although our calculations require a number of assumptions, we may check these
579 estimates against available empirical data in the literature. If we assume the copepod
580 population in Otsuchi Bay was in steady state, the production rate that corresponds to the
581 observed fraction of dead copepods would be ca. 0.08 d^{-1} . This is very comparable to the
582 global temperature-dependent marine copepod production rate established by Huntley and
583 Lope (1992) (ca. 0.1 d^{-1} at the *in situ* temperature). Furthermore, Ivory *et al.* (2014) used
584 sediment traps to quantify sinking copepod carcasses in Otsuchi Bay during the same

585 sampling period, and reported an average carcass carbon flux of $6.6 \text{ mg C m}^{-2} \text{ d}^{-1}$. This is also
586 of comparable magnitude to our carcass carbon export estimate on a per day basis, and
587 suggests that 89% of the available export carcass carbon would reach the seafloor, with the
588 remaining 11% potentially lost to necrophagy within the water column (Elliott *et al.*, 2010;
589 Dubovskaya *et al.*, 2015).

590 Overall, our study showed that copepod carcasses were ubiquitous in Japanese coastal
591 waters, indicative of non-predation mortality processes that appeared to be temperature
592 dependent. On average about 49.5% of the carcass carbon would be remineralised within the
593 water column via microbial respiration, with the remainder potentially be exported to the
594 seafloor. In the present study, we used a 200- μm net, which would have under-sampled the
595 younger stages (e.g., nauplii and early copepodites). Proper quantification of mortality rates
596 and analysis of population dynamics as influenced by non-predation mortality would require
597 the use of different mesh sizes to capture all life stages (e.g. Elliott and Tang, 2011a).
598 Continuous warming along the Japanese coasts is expected to not only increase non-predation
599 mortality (hence the prevalence of zooplankton carcasses) but also alter microbial
600 decomposition rate of the carcasses, with the potential to change the overall structure and
601 functioning of the ecosystem. Further research into the fate of zooplankton carcasses in
602 Japanese coasts under a warming ocean scenario is needed.

603

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736 mesoplankton. *Journal of Plankton Research*, 34: 493–509.
- 737

738 **Table 1.** Sampling locations, sampling depths, sampling dates (dd.mm) and number of
 739 samples (n) for this study.

Location	Lat. (°N)	Long. (°E)	Mean depth (m)	Sampling dates	n
Seto Inland Sea	34.08	133.29	20.2	24–25.06	5
Hamanako estuary	34.73	137.58	8.9	17.06	3
				11–12.07	3
Sagami Bay	35.21	139.41	10.0	10–14.06	12
Tokyo Bay	35.55	139.90	26	03.06	1
Otsuchi Bay	39.34	141.93	32.1	27–31.05	8
				01–05.07	8

740

741

742 **Table 2.** Percent compositions of different copepod species within each of the three Orders of
 743 Calanoida, Cyclopoida and Harpacticoida. Only the species that accounted for at least 5% of
 744 the total numerical abundances were shown.

		Seto Inland Sea	Hamanako estuary (June) (July)		Sagami Bay	Tokyo Bay	Otsuchi Bay (May) (July)	
Calanoid	<i>Acartia omorii</i>	11.7	28.2	42.2	24.4	45.5		
	<i>Acartia steueri</i>				15.0			
	<i>Acartia hudsonica</i>						25.1	14.0
	<i>Acartia pacifica</i>		7.6					
	<i>Acartia sinjiensis</i>			12.5				
	<i>Paracalanus parvus</i>	40.1		11.0	11.2			8.8
	<i>Clausocalanus pergens</i>							14.8
	<i>Pseudocalanus newmani</i>							5.3
	<i>Acartia</i> copepodite	7.8	20.6	13.3	24.2	49.7	13.5	
	<i>Paracalanus</i> copepodite	36.4	35.8	8.7	17.0			12.4
	<i>Clausocalanus</i> copepodite							10.3
	<i>Pseudocalanus</i> copepodite						47.3	21.6
	Cyclopoid	<i>Oncaea scottidicarloi</i>					12.5	
<i>Oncaea venustra</i>					6.7			
<i>Oithona davisae</i>			25.6	55.2		37.5		
<i>Oithona similis</i>		9.6			12.5		22.6	9.2
<i>Oithona atlantica</i>							25.0	19.5
<i>Corycaeus affinis</i>		31.6		11.2	18.3			24.2
<i>Oncaea</i> copepodite					13.3			
<i>Oithona</i> copepodite			46.1	29.3	13.6	50.0	50.9	39.6
<i>Corycaeus</i> copepodite		58.8			8.8			
Harpacticoid	<i>Euterpina acutifrons</i>		54.6	81.8	60.3			
	<i>Microsetella norvegica</i>				11.5			21.4
	<i>Microsetella rosea</i>							38.6
	<i>Tigriopus japonicus</i>						100.0	17.2
	Harpacticoid copepodite		31.7		9.7			

745

746

747 **Table 3.** Copepod abundances for the main genera (ind m⁻³; mean±sd); np = not present

Location	<i>Acartia</i>	<i>Paracalanus</i>	<i>Oithona</i>	<i>Pseudocalanus</i>
Seto Inland Sea	315.4 ± 317.4	1346.7 ± 259.7	24.5 ± 15.8	np
Hamanako estuary	869.3 ± 869.5	211.4 ± 195.6	686.4 ± 673.9	np
Sagami Bay	4117.9 ± 4134.6	821.7 ± 516.3	145.8 ± 140.4	np
Tokyo Bay	2651.9	117.9	103.1	np
Otsuchi Bay	1443.0 ± 2205.5	234.7 ± 281.4	1355.9 ± 1977.5	2301.1 ± 2549.2
Global average	1861.5	536.5	463.1	2301.1

748

749

750 **Table 4.** Fractions of dead copepods for the main genera (%; mean±sd); np = not present

Location	<i>Acartia</i>	<i>Paracalanus</i>	<i>Oithona</i>	<i>Pseudocalanus</i>
Seto Inland Sea	9.4 ± 12.0	5.4 ± 10.7	6.7 ± 14.9	np
Hamanako estuary	29.2 ± 21.2	13.3 ± 13.9	53.4 ± 37.6	np
Sagami Bay	6.7 ± 10.8	3.2 ± 5.9	7.3 ± 10.9	np
Tokyo Bay	0.4	0	0	np
Otsuchi Bay	2.5 ± 3.3	0	23.1 ± 19.8	6.4 ± 7.4
Global average	9.6	4.4	18.1	6.4

751

752

753 **Table 5.** Copepod carcass carbon concentrations for the main genera ($\mu\text{g C m}^{-3}$; mean \pm sd);

754 np = not present

Location	<i>Acartia</i>	<i>Paracalanus</i>	<i>Oithona</i>	<i>Pseudocalanus</i>
Seto Inland Sea	41.2 \pm 52.5	48.1 \pm 90.4	0.5 \pm 1.2	np
Hamanako estuary	467.5 \pm 789.3	14.9 \pm 8.1	34.8 \pm 26.9	np
Sagami Bay	105.2 \pm 336.8	34.0 \pm 71.12	4.8 \pm 7.1	np
Tokyo Bay	21.3	0	0	np
Otsuchi Bay	104.6 \pm 308.3	0	128.6 \pm 175.6	250.1 \pm 606.5
Global average	148.0	19.4	33.7	250.1

755

756

757 **Table 6.** Different estimated parameters for carcass decomposition at the sampling locations.
 758 Mean depths are given in Table 1; mean water temperatures are given in Figure 2. See text
 759 for explanation.

Location	Carcass decomposition rate (% h ⁻¹)	Summed carcass carbon conc. (µg C m ⁻³)	Carcass carbon lost to microbial respiration (%)	Carcass carbon to seafloor (mg C m ⁻²)
Seto Inland Sea	11.7	89.8	58.0	0.77
Hamanako estuary	16.2	517.2	33.8	3.05
Sagami Bay	13.1	144.0	31.2	0.99
Tokyo Bay	11.5	21.3	72.4	0.15
Otsuchi Bay	6.4	483.3	51.9	7.46
Global average	11.8	251.1	49.5	2.48

760

761 **Figure captions**

762

763 Figure 1. Sampling locations for the present study. White circles indicate the coastal areas
764 investigated in this study; black dots indicate sampling points in each area.

765

766 Figure 2. Water temperature, salinity, dissolved oxygen concentration and chlorophyll a
767 concentration at the sampling locations (mean + s.d.). ND = no data due to malfunction of the
768 sensor.

769

770 Figure 3. Carcass carbon concentrations ($\mu\text{g C m}^{-3}$) for the main copepod genera (*Acartia*,
771 *Paracalanus* and *Oithona*) at the different sampling locations (TB = Tokyo Bay).
772 *Pseudocalanus* carcass carbon data are shown for Otsuchi Bay only. Each bar represents one
773 sample. For Hamanako estuary and Otsuchi Bay, the different sampling months are indicated
774 at the top. Note y-axes are in log scale.

775

776 Figure 4. Dissolve oxygen concentrations in carcass decomposition experiment for the
777 treatment vials (with copepod carcasses) and control vials (without carcasses). Data are
778 normalized to the respective initial values to aid comparison.

779

780 Figure 5. Fractions of dead copepods (%) in relation to chlorophyll *a* concentration, salinity,
781 D.O. concentration and temperature for the three main genera. Lines are regression functions
782 with r^2 values indicated (significant at $p < 0.05$).

783









